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August 28, 2000

Attorney Docket No.: 07039-220001

Box Patent Application
Commissioner for Patents
Washington, DC 20231

Presented for filing is a new continuation-in-part patent application of:

Applicant: LIEPING CHEN

Title: B7-H1, A NOVEL IMMUNOREGULATORY MOLECULE

Enclosed are the following papers, including those required to receive a filing date under 37 CFR 1.53(b):

	<u>Pages</u>
Specification	42
Claims	5
Abstract	1
Declaration	2 (unsigned)
Drawing(s)	24

Enclosures:

- Postcard;
- Copy of Small Entity Statement filed in the parent application.

This application is a continuation-in-part (and claims the benefit of priority under 35 USC 120) of U.S. application serial no. 09/451,291, filed November 30, 1999. The disclosure of the prior application is considered part of (and is incorporated by reference in) the disclosure of this application.

CERTIFICATE OF MAILING BY EXPRESS MAIL

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Date of Deposit August 28, 2000

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Peter Consoli

Peter Consoli

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August 28, 2000

Page 2

This application is entitled to small entity status. A copy of the small entity statement filed in the parent application, U.S. Serial No. 09/451,291, is included.

Basic filing fee	\$345
Total claims in excess of 20 times \$9	\$252
Independent claims in excess of 3 times \$39	\$0
Fee for multiple dependent claims	\$0
Total filing fee:	\$597

A check for the filing fee is enclosed. Please apply any other required fees or any credits to deposit account 06-1050, referencing the attorney docket number shown above.

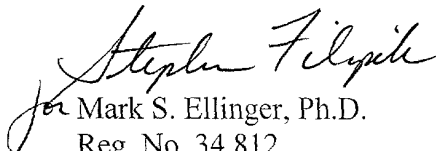
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Kindly acknowledge receipt of this application by returning the enclosed postcard.

Please send all correspondence to:

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Respectfully submitted,

 REG. No. 33,384
for Mark S. Ellinger, Ph.D.
Reg. No. 34,812
Enclosures
MSE/ptr
30025734 doc

Applicant or Patentee: Lieping Chen
 Serial or Patent No.: 09/451,291
 Filed or Issued: November 30, 1999
 For: B7-H1, A NOVEL IMMUNOREGULATORY MOLECULE

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
 (37 CFR 1.9(f) and 1.27(d)) — NONPROFIT ORGANIZATION**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

Name of Organization: MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH
 Address of Organization: 200 First Street S.W.
Rochester, MN 55905

Type of Organization:

- ☐ University or Other Institution of Higher Education
☒ Tax Exempt Under Internal Revenue Service Code (26 SC 501(a) and 501(c)(3))
☐ Nonprofit Scientific or Educational Under Statute of State of the United States of America
 (Name of State: _____) (Citation of Statute: _____)
☐ Would qualify as tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3)) if located in the
 United States of America
☐ Would qualify as nonprofit scientific or educational under Statute of State of the United States of America if located in
 the United States of America
 (Name of State: _____) (Citation of Statute: _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled B7-H1, A NOVEL IMMUNOREGULATORY MOLECULE by inventor(s) LIEPING CHEN described in:

- ☐ the specification filed herewith.
☒ application serial no. 09/451,291, filed November 30, 1999.
☐ patent no. , issued .

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

Full Name: _____
 Address: _____
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status when any new rule 53 application is filed or prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name:	Rick F. Colvin
Title:	Assistant Treasurer
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Signature: _____

Rick F. Colvin

Date: _____

2/7/2000

11/11/2000 10:00 AM Rick F. Colvin

CONTINUATION-IN-PART APPLICATION
FOR
UNITED STATES LETTERS PATENT

TITLE: B7-H1, A NOVEL IMMUNOREGULATORY MOLECULE
APPLICANT: LIEPING CHEN

CERTIFICATE OF MAILING BY EXPRESS MAIL

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Date of Deposit August 28, 2000

Signature Peter Consoli

Typed or Printed Name of Person Signing Certificate Peter Consoli

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled B7-H1, A NOVEL IMMUNOREGULATORY MOLECULE, the specification of which:

☒ is attached hereto.

☐ was filed on _ as Application Serial No. _ and was amended on _____.

☐ was described and claimed in PCT International Application No. _____ filed on _____ and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

<u>U.S. Serial No.</u>	<u>Filing Date</u>	<u>Status</u>
09/451,291	November 30, 1999	Pending

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Full Name of Inventor: LIEPING CHEN

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B7-H1, A NOVEL IMMUNOREGULATORY MOLECULE

This application claims priority of U.S. Patent Application No. 09/451,291, filed November 30, 1999.

BACKGROUND OF THE INVENTION

5 The invention is generally in the field of immunoregulation, and specifically T cell response regulation.

Mammalian T lymphocytes recognize antigenic peptides bound to major histocompatibility complex (MHC) molecules on the surface of antigen presenting cells (APC). The antigenic peptides are generated by proteolytic degradation of protein antigens within the APC. The interaction of the T cells with the APC and the subsequent response of the T cells are qualitatively and quantitatively regulated by interactions between cell surface receptors on the T cells with both soluble mediators and ligands on the surface of APC.

SUMMARY OF THE INVENTION

15 The invention is based on the cloning of human and mouse cDNA molecules encoding novel homologous molecules that co-stimulate the T cell responses of both species and on the functional characterization of the polypeptides that the cDNA molecules encode. The human polypeptide is designated hB7-H1 and the mouse polypeptide mB7-H1. Text that refers to B7-H1 without specifying human versus mouse is pertinent to both forms of B7-H1.

20 The invention features DNA molecules encoding the hB7-H1, mB7-H1 polypeptides, functional fragments of the polypeptides, and fusion proteins containing the polypeptides or functional fragments of the polypeptides, hB7-H1 and mB7-H1 and functional fragments of both, vectors containing the DNA molecules, and cells containing the vectors. Also included in the invention are antibodies that bind to the B7-H1 polypeptides. The invention features *in vitro*, *in vivo*, and *ex vivo* methods of co-stimulating T cell responses, methods of screening for compounds that inhibit or enhance T cell responses, and methods for producing the above polypeptides and fusion proteins.

25 Specifically the invention features an isolated DNA including: (a) a nucleic acid sequence that (i) encodes a B7-H1 polypeptide with the ability to co-stimulate a T cell, and (ii) hybridizes under stringent conditions to the complement of a sequence that encodes a polypeptide with an amino acid sequence with SEQ ID NO:1 or SEQ ID NO:3; or (b) a complement of this nucleic acid sequence. The nucleic acid sequence included in the

isolated DNA will be at least 10 bp, 15 bp, 25 bp, 50 bp, 75 bp, 100 bp, 125 bp, 150 bp, 175 bp, 200 bp, 250 bp, 300 bp, 350 bp, 400 bp, 450 bp, 500 bp, 550 bp, 600 bp, 650 bp, 700 bp, 750, bp 800 bp, 850 bp, or 870 bp long. The nucleic acid sequence can encode a B7-H1 polypeptide that includes an amino sequence with SEQ ID NO:1 or SEQ ID NO:3 or it can have a nucleotide sequences with SEQ ID NO:2 or SEQ ID NO:4. The nucleic acid sequence can also encode functional fragments of these B7-H1 polypeptides.

The invention also embodies an isolated B7-H1 polypeptide encoded by a DNA that includes a nucleic acid sequence that (i) encodes a polypeptide with the ability to co-stimulate a T cell and (ii) hybridizes under stringent conditions to the complement of a sequence that encodes a polypeptide with an amino acid sequence with SEQ ID NO:1 or SEQ ID NO:3. The B7-H1 polypeptide can include an amino sequence of amino acid residue 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, or 32, to amino acid residue 290 of SEQ ID NO:1 or SEQ ID NO:3. The invention also encompasses B7-H1 polypeptides that include an amino acid sequence with SEQ ID NO:1 or SEQ ID NO:3, or either of these amino acid sequences but differing solely by one or more conservative substitutions. The polypeptides of the invention include fusion proteins containing a first domain and at least one additional domain. The first domain can be any of the B7-H1 polypeptides described above or a functional fragment of any of these polypeptides. The at least one additional domain can be a heterologous targeting or leader sequence, an amino acid sequence that facilitates purification, detection, or solubility of the fusion protein. The second domain can be, for example, all or part of an immunoglobulin (Ig) heavy chain constant region. Also included are isolated nucleic acid molecules encoding the fusion proteins.

The invention features vectors containing any of the DNAs of the invention and nucleic acid molecules encoding the fusion proteins of the invention. The vectors can be expression vectors in which the nucleic acid coding sequence or molecule is operably linked to a regulatory element which allows expression of the nucleic acid sequence or molecule in a cell. Also included in the invention are cells (e.g., mammalian, insect, yeast, fungal, or bacterial cells) containing any of the vectors of the invention.

Another embodiment of the invention is a method of co-stimulating a T cell that involves contacting the T cell with any of the B7-H1 polypeptides of the invention, functional fragments thereof, or fusion proteins of the invention; these 3 classes of molecule are, for convenience, designated "B7-H1 agents". The contacting can be by culturing any of

these B7-H1 agents with the T cell *in vitro*. Alternatively, the T cell can be in a mammal and the contacting can be, for example, by administering any of the B7-H1 agents to the mammal or administering a nucleic acid encoding the B7-H1 agent to the mammal. In addition, the method can be an *ex vivo* procedure that involves providing a recombinant cell which is the

5 progeny of a cell obtained from the mammal and has been transfected or transformed *ex vivo* with a nucleic acid encoding any of the B7-H1 agents so that the cell expresses the B7-H1 agent; and administering the cell to the mammal. In this *ex vivo* procedure, the cell can be an antigen presenting cell (APC) that expresses the B7-H1 agent on its surface. Furthermore, prior to administering to the mammal, the APC can be pulsed with an antigen or an antigenic

10 peptide. In any of the above methods, the mammal can be suspected of having, for example, an immunodeficiency disease, an inflammatory condition, or an autoimmune disease. In addition, in any of the methods, the T cell can be a helper T cell, e.g., a T cell that helps an effector (e.g., a cytotoxic T lymphocyte (CTL) or B cell antibody) response. An antibody response can be, for example, an IgM, IgG1, IgG2a, IgG2b, IgG3, IgG4, IgE, or IgA

15 antibody response. Co-stimulation of a T cell by any of the B7-H1 agents can result in an increase in the level of CD40 ligand on the surface of the T cell.

The invention includes a method of identifying a compound that inhibits an immune response. The method involves: providing a test compound; culturing, together, the compound, one or more B7-H1 agents, a T cell, and a T cell activating stimulus; and

20 determining whether the test compound inhibits the response of the T cell to the stimulus, as an indication that the test compound inhibits an immune response. The invention also embodies a method of identifying a compound that enhances an immune response. The method involves: providing a test compound; culturing, together, the compound, one or more of B7-H1 agents, a T cell, and a T cell activating stimulus; and determining whether the test

25 compound enhances the response of the T cell to the stimulus, as an indication that the test compound enhances an immune response. In both these methods, the stimulus can be, for example, an antibody that binds to a T cell receptor or a CD3 polypeptide. Alternatively, the stimulus can be an alloantigen or an antigenic peptide bound to a major histocompatibility complex (MHC) molecule on the surface of an antigen presenting cell (APC). The APC can

30 be transfected or transformed with a nucleic acid encoding the B7-H1 agent and the B7-H1 agent can be expressed on the surface of the APC.

The invention also features an antibody (e.g., a polyclonal or a monoclonal antibody) that binds to any of the B7-H1 polypeptides of the invention, e.g., the polypeptide with SEQ ID NO:1 or SEQ ID NO:3.

The invention also features a method of producing any of the B7-H1 polypeptides of the invention, functional fragments thereof, or fusion proteins of the invention. The method involves culturing a cell of the invention and purifying the relevant B7-H1 protein from the culture.

"Polypeptide" and "protein" are used interchangeably and mean any peptide-linked chain of amino acids, regardless of length or post-translational modification. The invention also features B7-H1 polypeptides with conservative substitutions. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine, glutamine, serine and threonine; lysine, histidine and arginine; and phenylalanine and tyrosine.

The term "isolated" polypeptide or peptide fragment as used herein refers to a polypeptide or a peptide fragment which either has no naturally-occurring counterpart (e.g., a peptidomimetic), or has been separated or purified from components which naturally accompany it, e.g., in tissues such as pancreas, liver, spleen, ovary, testis, muscle, joint tissue, neural tissue, gastrointestinal tissue, or body fluids such as blood, serum, or urine. Typically, the polypeptide or peptide fragment is considered "isolated" when it is at least 70%, by dry weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, a preparation of a polypeptide (or peptide fragment thereof) of the invention is at least 80%, more preferably at least 90%, and most preferably at least 99%, by dry weight, the polypeptide (or the peptide fragment thereof), respectively, of the invention. Thus, for example, a preparation of polypeptide x is at least 80%, more preferably at least 90%, and most preferably at least 99%, by dry weight, polypeptide x. Since a polypeptide that is chemically synthesized is, by its nature, separated from the components that naturally accompany it, the synthetic polypeptide or nucleic acid is "isolated."

An isolated polypeptide (or peptide fragment) of the invention can be obtained, for example, by extraction from a natural source (e.g., from human tissues or bodily fluids); by expression of a recombinant nucleic acid encoding the peptide; or by chemical synthesis. A peptide that is produced in a cellular system different from the source from which it

naturally originates is "isolated," because it will be separated from components which naturally accompany it. The extent of isolation or purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

5 An "isolated DNA" means DNA free of one or both of the genes that flank the gene containing the DNA of interest in the genome of the organism in which the gene containing the DNA of interest naturally occurs. The term therefore includes a recombinant DNA incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote. It also includes a separate molecule such as:

10 a cDNA where the corresponding genomic DNA has introns and therefore a different sequence; a genomic fragment; a fragment produced by polymerase chain reaction (PCR); a restriction fragment; a DNA encoding a non-naturally occurring protein, fusion protein, or fragment of a given protein; or a nucleic acid which is a degenerate variant of a naturally occurring nucleic acid. In addition, it includes a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Also included is a recombinant DNA that includes a portion of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:5. It will be apparent from the foregoing that isolated DNA does not mean a DNA present among hundreds to millions of other DNA molecules within, for example, cDNA or genomic DNA libraries or genomic DNA restriction digests in, for example, a restriction digest reaction mixture or an

15 electrophoretic gel slice.

20

As used herein, a polypeptide that "co-stimulates" a T cell is a polypeptide that, upon interaction with a cell-surface molecule on the T cell, enhances the response of the T cell. The T cell response that results from the interaction will be greater than the response in the absence of the polypeptide. The response of the T cell in the absence of the co-stimulatory polypeptide can be no response or it can be a response significantly lower than in the

25 presence of the co-stimulatory polypeptide. It is understood that the response of the T cell can be an effector (e.g., CTL or antibody-producing B cell) response, a helper response providing help for one or more effector (e.g., CTL or antibody-producing B cell) responses, or a suppressive response.

30 As used herein, an "activating stimulus" is a molecule that delivers an activating signal to a T cell, preferably through the antigen specific T cell receptor (TCR). The activating stimulus can be sufficient to elicit a detectable response in the T cell.

Alternatively, the T cell may require co-stimulation (e.g., by a B7-H1 polypeptide) in order to respond detectably to the activating stimulus. Examples of activating stimuli include, without limitation, antibodies that bind to the TCR or to a polypeptide of the CD3 complex that is physically associated with the TCR on the T cell surface, alloantigens, or an antigenic peptide bound to a MHC molecule.

As used herein, a "fragment" of a B7-H1 polypeptide is a fragment of the polypeptide that is shorter than the full-length polypeptide. Generally, fragments will be five or more amino acids in length. An antigenic fragment has the ability to be recognized and bound by an antibody.

As used herein, a "functional fragment" of a B7-H1 polypeptide is a fragment of the polypeptide that is shorter than the full-length polypeptide and has the ability to co-stimulate a T cell. Methods of establishing whether a fragment of an B7-H1 molecule is functional are known in the art. For example, fragments of interest can be made by either recombinant, synthetic, or proteolytic digestive methods. Such fragments can then be isolated and tested for their ability to co-stimulate T cells by procedures described herein.

As used herein, "operably linked" means incorporated into a genetic construct so that expression control sequences effectively control expression of a coding sequence of interest.

As used herein, the term "antibody" refers not only to whole antibody molecules, but also to antigen-binding fragments, e.g., Fab, F(ab')₂, Fv, and single chain Fv fragments. Also included are chimeric antibodies.

As used herein, an antibody that "binds specifically" to an isolated B7-H1 polypeptide encoded by a DNA that includes a nucleic acid sequence that (i) encodes a polypeptide with the ability to co-stimulate a T cell and (ii) hybridizes under stringent conditions to the complement of a sequence that encodes a polypeptide with an amino acid sequence with SEQ ID NO:1 or SEQ ID NO:3, is an antibody that does not bind to B7-1 or B7-2 polypeptides.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. In case of conflict, the present document, including definitions, will control.

Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention. All publications, patent applications, patents and other references mentioned

herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

Other features and advantages of the invention, e.g., enhancing immune responses in mammalian subjects, will be apparent from the following description, from the drawings and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a depiction of the nucleotide sequence of a cDNA fragment (SEQ ID NO: 5) that includes the coding sequence (nucleotides 72-870 of SEQ ID NO:5) (SEQ ID NO:2) of hB7-H1.

FIG. 2a is a depiction of the amino acid sequence of hB7-H1 (SEQ ID NO:1). The signal peptide, Ig-V-like domain, Ig-C-like domain, and transmembrane ("TM") domain are indicated. Potential N-linked glycosylation sites are indicated by *.

FIG. 2b is a depiction of the amino acid sequences of the extracellular domains of hB7-H1 (SEQ ID NO:10), human B7-1 (hB7-1; SEQ ID NO:11), and human B7-2 (hB7-2; SEQ ID NO:12) aligned for maximum homology. Identical amino acid residues are shaded in bold and conserved residues are boxed. Conserved cysteine residues are indicated by *.

FIG. 3 is a photograph of a Northern blot showing expression of hB7-H1 mRNA in various human tissues.

FIG. 4 is a series of two-dimensional fluorescence flow cytometry histograms showing cell surface expression of hB7-H1 on resting and activated CD3+ T cells, CD19+ B cells, and CD14+ monocytes.

FIG. 5a is a series of fluorescence flow cytometry histograms showing binding of CTLA-4Ig, ICOSIg, and antibody specific for hB7-H1 to 293 cells transfected with either a control vector ("Mock/293 cells") or a vector containing a cDNA insert encoding hB7-H1 ("hB7-H1/293 cells"), or Raji cells.

FIG. 5b is a series of fluorescence flow cytometry histograms showing the binding of hB7-H1Ig and antibody to CD28 to Jurkat cells.

FIG. 6a is a line graph showing the ability of immobilized hB7-H1Ig to co-stimulate the proliferative response of human T cells to immobilized antibody specific for human CD3.

FIG. 6b is a line graph showing the ability of soluble hB7-H1Ig to co-stimulate the proliferative response of human T cells to irradiated allogeneic PBMC.

FIGS. 7a-7d are a series of line graphs showing the ability of hB7-H1Ig, human B7-1Ig, or antibody specific for human CD28 to co-stimulate the production of interleukin-10 (FIG. 7a), interferon- γ (IFN- γ) (FIG. 7b), IL-2 (FIG. 7c), or IL-4 (FIG. 7d) by human T cells responding to immobilized antibody specific for CD3. FIG. 7e is a line graph showing the ability of various concentrations of hB7-H1Ig to co-stimulate the production of IL-2 by human T cells responding to immobilized antibody specific for CD3.

FIG. 8a is a bar graph showing the ability of antibody specific for human IL-2 to inhibit the proliferation of human T cells induced by antibody specific for human CD3 and co-stimulated by COS cells transfected with and expressing either hB7-H1 or human B7-1.

FIG. 8b is a bar graph showing the ability of antibody specific for human IL-2 to inhibit the production of IL-10 by human T cells stimulated by immobilized antibody specific for human CD3 and co-stimulated by either hB7-H1Ig or B7-1Ig.

FIG. 9a is a series of two-dimensional fluorescence flow cytometry profiles showing the relative proportion of T cells in early (annexin V-positive, propidium iodide (PI)-negative) and late (annexin V-positive, PI-positive) apoptosis following activation by immobilized antibody specific for human CD3 and co-stimulation with either control Ig or hB7-H1Ig.

FIG. 9b is series of fluorescence flow cytometry profiles showing expression of Fas and FasL on human T cells following activation by immobilized antibody specific for human CD3 and co-stimulation with either control Ig or hB7-H1Ig.

FIG. 10 is a depiction of the nucleotide sequence of cDNA encoding mB7-H1 (SEQ ID NO:4).

FIG. 11 is a depiction of the amino acid sequence of mB7-H1 (SEQ ID NO:3).

FIG. 12a is a depiction of the amino acid sequence of mB7-H1 (SEQ ID NO:3) aligned with the amino acid sequence of hB7-H1 (SEQ ID NO:1). The signal peptide, IgV-like domain, IgC-like domain, transmembrane ("TM") domain, and cytoplasmic domain ("cytoplasmic") of mB7-H1 are indicated.

FIG. 12b is a depiction of the amino acid sequences of mB7-H1, mouse B7-1 (mB7-1; SEQ ID NO:13), mouse B7-2 (mB7-2; SEQ ID NO:14), and mB7h/B7KF-1 (SEQ ID NO:15) aligned for maximum homology. Identical amino acid residues are shaded and conserved amino acid residues are boxed. Conserved cysteine residues are indicated by *.

FIG. 13 is a series of fluorescence flow cytometry histograms showing relative levels of cell surface expression of mB7-H1 on resting and activated mouse CD3+ T cells, mouse B220+ B cells, and Mac-1+ mouse macrophages.

FIG. 14a is a line graph showing the ability of various concentrations of immobilized mB7-H1Ig or immobilized control Ig to co-stimulate the *in vitro* proliferative response of mouse T cells to a suboptimal dose of immobilized antibody specific for mouse CD3.

FIG. 14b is a pair of line graphs showing the ability of immobilized mB7-H1Ig, immobilized control Ig, or soluble antibody specific for mouse CD28 to co-stimulate the *in vitro* proliferative response of wild type ("wt") C57BL/6 mouse (left graph) or CD28-deficient ("CD28^{-/-}") C57BL/6 mouse (right graph) T cells to two suboptimal doses of immobilized antibody specific for mouse CD3.

FIG. 14c is a bar graph showing the ability of immobilized mB7-H1Ig or immobilized control Ig to co-stimulate the *in vitro* proliferative response of purified CD4+ or CD8+ mouse T cells to a suboptimal dose of immobilized antibody specific for mouse CD3.

FIG. 15a is a series of line graphs showing the ability of immobilized mB7-H1Ig, immobilized control Ig, or soluble antibody specific for mouse CD28 ("Anti-CD28") to co-stimulate the *in vitro* production (on days one, two, and three after initiation of the cultures) of various cytokines by C57BL/6 mouse T cells in response to immobilized antibody specific for mouse CD3.

FIG. 15b is a line graph showing the effect of immobilized mB7-H1Ig on the *in vitro* production (18, 24, 36, and 48 hours after initiation of the cultures) of IL-2 by C57BL/6 mouse T cells responding to immobilized antibody specific for mouse CD3 and co-stimulated by soluble antibody specific for mouse CD28 ("Anti-CD28").

FIG. 16a and FIG. 16b are fluorescence flow cytometry histograms showing lack of surface expression of murine B7-1 (FIG. 16a) and mB7-H1 (FIG. 16b) on P815 cells transfected with a control expression vector ("mock.P815").

FIG. 17a and FIG. 17b are fluorescence flow cytometry histograms showing surface expression of murine B7-1 (FIG. 17a) and lack of surface expression of mB7-H1 (FIG. 17b) on P815 cells transfected with an expression vector containing a nucleic acid sequence encoding murine B7-1 ("mB7-1⁺ P815").

FIG. 18a and FIG. 18b are fluorescence flow cytometry histograms showing lack of surface expression of mB7-1 (FIG. 18a) and showing surface expression of mB7-H1 (FIG.

18b) on P815 cells transfected with an expression vector containing a nucleic acid sequence encoding mB7-H1 ("mB7-H1⁺ P815").

FIG. 19a and FIG. 19b are line graphs showing the growth rate of P815 tumors in DBA/2 mice injected subcutaneously with P815 cells transfected with a control expression vector (FIG. 19a) or an expression vector containing a nucleic acid sequence encoding mB7-H1 (FIG. 19b).

FIG. 20a is a pair of line graphs showing the ability of control mock-transfected P815 cells ("mock. P815"), P815 cells transfected with an expression vector containing a cDNA sequence encoding mB7-H1 ("mB7-H1⁺ P815"), or P815 cells transfected with an expression vector containing a cDNA sequence encoding mB7-1 ("mB7-1⁺ P815") to activate allospecific C57BL/6 mouse CTL *in vitro*. The effector cell populations were tested for cytotoxic activity ("% Lysis") at various effector to target cell (E/T) ratios against wild type P815 (left graph) and control EL4 (right graph) target cells.

FIG. 20b is a pair of line graphs showing the ability of control mock-transfected P815 cells ("mock. P815"), P815 cells transfected with an expression vector containing a cDNA sequence encoding mB7-H1 ("mB7-H1⁺ P815"), or P815 cells transfected with an expression vector containing a cDNA sequence encoding mB7-1 ("mB7-1⁺ P815") to activate tumor-specific DBA/2 mouse CTL *in vivo*. The effector cell populations were tested for cytotoxic activity ("% Lysis") at various E/T ratios against wild type P815 (left graph) and control L1210 (right graph) target cells.

FIG. 21 is a pair of line graphs showing the *in vitro* proliferative responses to various concentrations of keyhole limpet hemacyanin (KLH) of draining lymph node (left graph) or spleen (right graph) T cells from C57BL/6 immunized intraperitoneally (i.p.) with trinitrophenol (TNP) conjugated KLH (TNP-KLH) in incomplete Freund's adjuvant and subsequently injected i.p. with mB7-H1Ig or control Ig.

FIG. 22 is a series of bar graphs showing the relative levels (as measured by ELISA) of IgG1, IgG2a, IgG2b, IgG3, or IgM antibodies specific for TNP in the sera of C57BL/6 mice injected i.p. with TNP-KLH in phosphate buffered saline and either control Ig, mB7-H1Ig, or mB7-1Ig.

FIG. 23a is a series of fluorescence flow cytometry histograms showing the relative levels of cell surface expression of CD40 ligand (CD40L) (as measured by the binding of an antibody specific for mouse CD40L ("anti-CD40L")) on purified mouse CD4⁺ T cells (at 4

and 24 hours after initiation of the cultures) activated by immobilized antibody specific for mouse CD3 ("anti-CD3") coated onto the bottoms of tissue culture wells at a concentration of 200 ng/ml and co-stimulated by immobilized control Ig, immobilized mB7-H1Ig, or soluble antibody specific for mouse CD28 ("anti-CD28").

FIG. 23b is a series of fluorescence flow cytometry histograms showing the relative levels of cell surface expression of CD40 ligand (CD40L) (as measured by the binding of an antibody specific for mouse CD40L ("anti-CD40L")) on purified mouse CD4+ T cells (at 4 and 24 hours after initiation of the cultures) activated by immobilized antibody specific for mouse CD3 ("anti-CD3") coated onto the bottoms of tissue culture wells at a concentration of 1,000 ng/ml and co-stimulated by immobilized control Ig, immobilized mB7-H1Ig, or soluble antibody specific for mouse CD28 ("anti-CD28").

DETAILED DESCRIPTION

Using PCR primers with sequences derived from an expressed sequence tag (EST) that had significant homology to human B7-1 and B7-2 and a human cDNA library as a source of template, cDNA sequences corresponding to regions of a transcript 5' and 3' of the EST were identified. A cDNA molecule (SEQ ID NO:5) that included a open reading frame (orf) (SEQ ID NO:2) encoding a novel B7-related molecule was then generated using PCR primers with sequences derived from the 3' and 5' ends and cloned.

Translation of the cDNA sequence indicated that the polypeptide (SEQ ID NO:1) that it encoded (hB7-H1) is a type I transmembrane protein of 290 amino acids containing an immunoglobulin (Ig) V-like domain, Ig C-like domain, a transmembrane domain and a cytoplasmic domain of 30 amino acids. Northern blot analysis showed strong expression of the gene encoding hB7-H1 in heart, skeletal muscle, placenta, and lung, and weak expression in thymus, spleen, kidney, and liver. Expression was undetectable in brain, colon, small intestine, and peripheral blood mononuclear cells (PBMC).

Using an antiserum produced by immunization of mice with a recombinantly produced fusion protein that included the hB7-H1 protein, expression by fluorescence flow cytometry indicated negligible expression on resting T and B cells. On the other hand, about 16% of CD14+ monocytes constitutively expressed the molecule on their surface. Activation of T cells increased expression such that about 30% expressed cell-surface hB7-H1.

Activation resulted in about 90% of monocytes expressing hB7-1H, but only about 6% of B cells expressed it after activation.

Transfection of 293 cells resulted in an hB7-H1 expressing cell line (hB7-H1/293) which was used for binding experiments. These experiments and others with a CD28 expressing cell line indicated that neither CTLA4, ICOS, nor CD28 were receptors for hB7-H1.

In vitro experiments with isolated human T cells and the hB7-H1-containing fusion protein indicated that hB7-H1 had no direct activity on T cells, it enhanced ("co-stimulated") T cell proliferative responses induced by both antibody specific for human CD3 and MHC alloantigens. This co-stimulatory activity was significantly more potent when the hB7-H1 was immobilized in the plastic tissue culture wells used for the cultures than when it as in solution. Similar experiments indicated that hB7-H1 had a dramatic and selective enhancing effect on the production of interleukin (IL)-10 induced by T cell activation. Moreover this IL-10 enhancing activity appeared to be dependent on at least low amounts of IL-2. Analysis of T cells activated by anti-CD3 antibody and hB7-H1Ig indicated that hB7-H1 enhances apoptosis and expression of Fas and FasL

In addition, using a strategy similar to that used to clone hB7-H1 cDNA, a cDNA molecule containing an orf encoding mouse B7-H1 (mB7-H1) was cloned, the nucleotide sequence of the orf (SEQ ID NO:4) was obtained, and the amino acid sequence of the encoded sequence (SEQ ID NO:3) was derived. mB7-H1 is exactly the same length (290 amino acids) and has the same domain structure as hB7-H1. Moreover, mB7-H1 has a similar tissue distribution to hB7-H1. mB7-H1 co-stimulated the response of mouse T cells with its effect being more potent on CD4+ than on CD8+ T cells. In addition, like hB7-H1, mB7-H1 co-stimulates the production of high levels of IL-10 by T cells. mB7-H1 also enhanced the production of both interferon- γ (IFN- γ) and granulocyte macrophage-colony stimulating factor (GM-CSF) by T cells. While mB7-H1 showed no significant ability to enhance CTL responses, it did greatly increase antibody responses and, in particular, IgG2a antibody responses. Finally, co-stimulation of T cells with mB7-H1 caused an increase in the level of CD40 ligand (CD40L) on the surface of the T cells.

B7-H1 can be useful as an augmentor of immune responses (e.g., helper T cell and antibody responses) both *in vivo* and *in vitro*. Furthermore, in light of (a) its ability to selectively enhance IL-10 production, (b) its ability to enhance apoptosis, and (c) is

expression in placenta and lung, both organs normally protected from unneeded cellular-mediated immune and inflammatory responses, B7-H1 can be useful in controlling pathologic cell-mediated conditions (e.g., those induced by infectious agents such *Mycobacterium tuberculosis* or *M. leprae*) or other pathologic cell-mediated responses such as those involved in autoimmune diseases (e.g., rheumatoid arthritis (RA), multiple sclerosis (MS), or insulin-dependent diabetes mellitus (IDDM)).

B7-H1 Nucleic Acid Molecules

The B7-H1 nucleic acid molecules of the invention can be cDNA, genomic DNA, synthetic DNA, or RNA, and can be double-stranded or single-stranded (*i.e.*, either a sense or an antisense strand). Segments of these molecules are also considered within the scope of the invention, and can be produced by, for example, the polymerase chain reaction (PCR) or generated by treatment with one or more restriction endonucleases. A ribonucleic acid (RNA) molecule can be produced by *in vitro* transcription. Preferably, the nucleic acid molecules encode polypeptides that, regardless of length, are soluble under normal physiological conditions the membrane forms would not be soluble.

The nucleic acid molecules of the invention can contain naturally occurring sequences, or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide (for example, the polypeptides with SEQ ID NOS:1 and 3). In addition, these nucleic acid molecules are not limited to coding sequences, e.g., they can include some or all of the non-coding sequences that lie upstream or downstream from a coding sequence. They include, for example, the nucleic acid molecule with SEQ ID NO:5.

The nucleic acid molecules of the invention can be synthesized (for example, by phosphoramidite-based synthesis) or obtained from a biological cell, such as the cell of a mammal. Thus, the nucleic acids can be those of a human, non-human primate (e.g., monkey) mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, dog, or cat.

In addition, the isolated nucleic acid molecules of the invention encompass segments that are not found as such in the natural state. Thus, the invention encompasses recombinant nucleic acid molecules, (for example, isolated nucleic acid molecules encoding hB7-H1 or mB7-H1) incorporated into a vector (for example, a plasmid or viral vector) or into the genome of a heterologous cell (or the genome of a homologous cell, at a position other than

the natural chromosomal location). Recombinant nucleic acid molecules and uses therefor are discussed further below.

Certain nucleic acid molecules of the invention are antisense molecules or are transcribed into antisense molecules. These can be used, for example, to down-regulate translation of B7-H1 mRNA within a cell.

Techniques associated with detection or regulation of genes are well known to skilled artisans and such techniques can be used to diagnose and/or treat disorders associated with aberrant B7-H1 expression. Nucleic acid molecules of the invention are discussed further below in the context of their therapeutic utility.

A B7-H1 family gene or protein can be identified based on its similarity to the relevant B7-H1 gene or protein, respectively. For example, the identification can be based on sequence identity. The invention features isolated nucleic acid molecules which are at least 50% (or 55%, 65%, 75%, 85%, 95%, or 98%) identical to: (a) a nucleic acid molecule that encodes the polypeptide of SEQ ID NO:1 or 3; (b) the nucleotide sequence of SEQ ID NO:2 or 4; or (c) a nucleic acid molecule which includes a segment of at least 30 (e.g., at least 50, 60, 100, 125, 150, 175, 200, 250, 300, 325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, or 865) nucleotides of SEQ ID NO:2 or SEQ ID NO:4.

The determination of percent identity between two sequences is accomplished using the mathematical algorithm of Karlin and Altschul, *Proc. Natl. Acad. Sci. USA* 90, 5873-5877, 1993. Such an algorithm is incorporated into the BLASTN and BLASTP programs of Altschul et al. (1990) *J. Mol. Biol.* 215, 403-410. BLAST nucleotide searches are performed with the BLASTN program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to B7-H1-encoding nucleic acids. BLAST protein searches are performed with the BLASTP program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to B7-H1. To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described in Altschul et al. (1997) *Nucleic Acids Res.* 25, 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) are used (See <http://www.ncbi.nlm.nih.gov>).

Hybridization can also be used as a measure of homology between two nucleic acid sequences. A B7-H1-encoding nucleic acid sequence, or a portion thereof, can be used as hybridization probe according to standard hybridization techniques. The hybridization of a

B7-H1 probe to DNA from a test source (e.g., a mammalian cell) is an indication of the presence of B7-H1 DNA in the test source. Hybridization conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., 6.3.1-6.3.6, 1991. Moderate hybridization conditions are defined as equivalent to hybridization in 2X sodium chloride/sodium citrate (SSC) at 30°C, followed by one or more washes in 1 X SSC, 0.1% SDS at 50-60°C. Highly stringent conditions are defined as equivalent to hybridization in 6X sodium chloride/sodium citrate (SSC) at 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C.

The invention also encompasses: (a) vectors that contain any of the foregoing B7-H1-related coding sequences and/or their complements (that is, "antisense" sequence); (b) expression vectors that contain any of the foregoing B7-H1-related coding sequences operatively associated with any transcriptional/translational regulatory elements (examples of which are given below) necessary to direct expression of the coding sequences; (c) expression vectors containing, in addition to sequences encoding a B7-H1 polypeptide, nucleic acid sequences that are unrelated to nucleic acid sequences encoding B7-H1, such as molecules encoding a reporter, marker, or a signal peptide, e.g., fused to B7-H1; and (d) genetically engineered host cells that contain any of the foregoing expression vectors and thereby express the nucleic acid molecules of the invention.

Recombinant nucleic acid molecules can contain a sequence encoding hB7-H1 or mB7-H1, or B7-H1 having an heterologous signal sequence. The full length B7-H1 polypeptide, a domain of B7-H1, or a fragment thereof may be fused to additional polypeptides, as described below. Similarly, the nucleic acid molecules of the invention can encode the mature form of B7-H1 or a form that includes an exogenous polypeptide which facilitates secretion.

The transcriptional/translational regulatory elements referred to above and which are further described below, include, but are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements, which are known to those skilled in the art, and which drive or otherwise regulate gene expression. Such regulatory elements include but are not limited to the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage A, the control regions of fd coat

protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α -mating factors.

Similarly, the nucleic acid can form part of a hybrid gene encoding additional polypeptide sequences, for example, sequences that function as a marker or reporter.

5 Examples of marker or reporter genes include β -lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r , G418^r), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β -galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT). As with many of the standard procedures associated
10 with the practice of the invention, skilled artisans will be aware of additional useful reagents, for example, additional sequences that can serve the function of a marker or reporter. Generally, the hybrid polypeptide will include a first portion and a second portion; the first portion being a B7-H1 polypeptide and the second portion being, for example, the reporter described above or an Ig constant region or part of an Ig constant region, e.g., the CH2 and
15 CH3 domains of IgG2a heavy chain.

The expression systems that may be used for purposes of the invention include, but are not limited to, microorganisms such as bacteria (for example, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expres-
20 sion vectors containing the nucleic acid molecules of the invention; yeast (for example, *Saccharomyces* and *Pichia*) transformed with recombinant yeast expression vectors containing the nucleic acid molecules of the invention (preferably containing the nucleic acid sequence encoding B7-H1 (contained within SEQ ID NOS:1 or 3); insect cell systems infected with recombinant virus expression vectors (for example, baculovirus) containing the nucleic acid molecules of the invention; plant cell systems infected with recombinant virus
25 expression vectors (for example, cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (for example, Ti plasmid) containing B7-H1 nucleotide sequences; or mammalian cell systems (for example, COS, CHO, BHK, 293, VERO, HeLa, MDCK, WI38, and NIH 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of
30 mammalian cells (for example, the metallothionein promoter) or from mammalian viruses (for example, the adenovirus late promoter and the vaccinia virus 7.5K promoter). Also

useful as host cells are primary or secondary cells obtained directly from a mammal, transfected with a plasmid vector or infected with a viral vector.

Polypeptides and Polypeptide Fragments

5 The polypeptides of the invention include hB7-H1, mB7-H1, and functional fragments of these polypeptides. The polypeptides embraced by the invention also include fusion proteins which contain either full-length B7-H1 or a functional fragment of it fused to unrelated amino acid sequence. The unrelated sequences can be additional functional domains or signal peptides. Signal peptides are described in greater detail and exemplified below. The polypeptides can be any of those described above but with one or more (e.g., 10 one, two, three, four, five, six, seven, eight, nine, 10, 12, 14, 17, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more) conservative substitutions.

The polypeptides can be purified from natural sources (e.g., blood, serum plasma, tissues or cells such as T cells or any cell that naturally produces B7-H1). Smaller peptides (less than 50 amino acids long) can also be conveniently synthesized by standard chemical means. In addition, both polypeptides and peptides can be produced by standard *in vitro* recombinant DNA techniques and *in vivo* recombination/genetic recombination (e.g., transgenesis), using the nucleotide sequences encoding the appropriate polypeptides or peptides. Methods well known to those skilled in the art can be used to construct expression vectors containing relevant coding sequences and appropriate transcriptional/translational control signals. See, for example, the techniques described in Sambrook et al., Molecular Cloning: A Laboratory Manual (2nd Ed.) [Cold Spring Harbor Laboratory, N.Y., 1989], and Ausubel et al., Current Protocols in Molecular Biology, [Green Publishing Associates and Wiley Interscience, N.Y., 1989].

25 Polypeptides and fragments of the invention also include those described above, but modified for *in vivo* use by the addition, at the amino- and/or carboxyl-terminal ends, of a blocking agent to facilitate survival of the relevant polypeptide *in vivo*. This can be useful in those situations in which the peptide termini tend to be degraded by proteases prior to cellular uptake. Such blocking agents can include, without limitation, additional related or 30 unrelated peptide sequences that can be attached to the amino and/or carboxyl terminal residues of the peptide to be administered. This can be done either chemically during the

synthesis of the peptide or by recombinant DNA technology by methods familiar to artisans of average skill.

Alternatively, blocking agents such as pyroglutamic acid or other molecules known in the art can be attached to the amino and/or carboxyl terminal residues, or the amino group at the amino terminus or carboxyl group at the carboxyl terminus can be replaced with a different moiety. Likewise, the peptides can be covalently or noncovalently coupled to pharmaceutically acceptable "carrier" proteins prior to administration.

Also of interest are peptidomimetic compounds that are designed based upon the amino acid sequences of the functional peptide fragments. Peptidomimetic compounds are synthetic compounds having a three-dimensional conformation (i.e., a "peptide motif") that is substantially the same as the three-dimensional conformation of a selected peptide. The peptide motif provides the peptidomimetic compound with the ability to co-stimulate T cells in a manner qualitatively identical to that of the B7-H1 functional peptide fragment from which the peptidomimetic was derived. Peptidomimetic compounds can have additional characteristics that enhance their therapeutic utility, such as increased cell permeability and prolonged biological half-life.

The peptidomimetics typically have a backbone that is partially or completely non-peptide, but with side groups that are identical to the side groups of the amino acid residues that occur in the peptide on which the peptidomimetic is based. Several types of chemical bonds, e.g., ester, thioester, thioamide, retroamide, reduced carbonyl, dimethylene and ketomethylene bonds, are known in the art to be generally useful substitutes for peptide bonds in the construction of protease-resistant peptidomimetics.

Methods of Co-stimulating a T Cell

The methods of the invention involve contacting a T cell with a B7-H1 polypeptide of the invention, or a functional fragment thereof, in order to co-stimulate the T cell. Such polypeptides or functional fragments can have amino acid sequences identical to wild-type sequences or they can contain one or more (e.g., one, two, three, four, five, six, seven, eight, nine, 10, 12, 14, 17, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more) conservative substitutions. The contacting can occur before, during, or after activation of the T cell. Contacting of the T cell with the B7-H1 polypeptide will preferably be at substantially the same time as activation. Activation can be, for example, by exposing the T cell to an

antibody that binds to the TCR or one of the polypeptides of the CD3 complex that is physically associated with the TCR. Alternatively, the T cell can be exposed to either an alloantigen (e.g., a MHC alloantigen) on, for example, an antigen presenting cell (APC) (e.g., a dendritic cell, a macrophage, a monocyte, or a B cell) or an antigenic peptide produced by processing of a protein antigen by any of the above APC and presented to the T cell by MHC molecules on the surface of the APC. The T cell can be a CD4⁺ T cell or a CD8⁺ T cell. The B7-H1 molecule can be added to the solution containing the cells, or it can be expressed on the surface of an APC, e.g., an APC presenting an alloantigen or an antigen peptide bound to an MHC molecule. Alternatively, if the activation is *in vitro*, the B7-H1 molecule can be bound to the floor of a the relevant culture vessel, e.g. a well of a plastic microtiter plate.

The methods can be performed *in vitro*, *in vivo*, or *ex vivo*. *In vitro* application of B7-H1 can be useful, for example, in basic scientific studies of immune mechanisms or for production of activated T cells for use in either studies on T cell function or, for example, passive immunotherapy. Furthermore, B7-H1 could be added to *in vitro* assays (e.g., in T cell proliferation assays) designed to test for immunity to an antigen of interest in a subject from which the T cells were obtained. Addition of B7-H1 to such assays would be expected to result in a more potent, and therefore more readily detectable, *in vitro* response. However, the methods of the invention will preferably be *in vivo* or *ex vivo* (see below).

The B7-H1 proteins and variants thereof are generally useful as immune response-stimulating therapeutics. For example, the polypeptides of the invention can be used for treatment of disease conditions characterized by immunosuppression: e.g., cancer, AIDS or AIDS-related complex, other virally or environmentally-induced conditions, and certain congenital immune deficiencies. The compounds may also be employed to increase immune function that has been impaired by the use of radiotherapy of immunosuppressive drugs such as certain chemotherapeutic agents, and therefore are particularly useful when given in conjunction with such drugs or radiotherapy. In addition, in view of the ability of B7-H1 to co-stimulate the production of especially high levels of IL-10, B7-H1 molecules can be used to treat conditions involving cellular immune responses, e.g., inflammatory conditions, e.g., those induced by infectious agents such *Mycobacterium tuberculosis* or *M. leprae*, or other pathologic cell-mediated responses such as those involved in autoimmune diseases (e.g., rheumatoid arthritis (RA), multiple sclerosis (MS), or insulin-dependent diabetes mellitus (IDDM)).

These methods of the invention can be applied to a wide range of species, e.g., humans, non-human primates, horses, cattle, pigs, sheep, goats, dogs, cats, rabbits, quinea pigs, hamsters, rats, and mice.

In Vivo Approaches

5 In one *in vivo* approach, the B7-H1 polypeptide (or a functional fragment thereof) itself is administered to the subject. Generally, the compounds of the invention will be suspended in a pharmaceutically-acceptable carrier (e.g., physiological saline) and administered orally or by intravenous infusion, or injected subcutaneously, intramuscularly, intraperitoneally, intrarectally, intravaginally, intranasally, intragastrically, intratracheally, or
10 intrapulmonarily. They are preferably delivered directly to an appropriate lymphoid tissue (e.g. spleen, lymph node, or mucosal-associated lymphoid tissue (MALT)). The dosage required depends on the choice of the route of administration, the nature of the formulation, the nature of the patient's illness, the subject's size, weight, surface area, age, and sex, other drugs being administered, and the judgment of the attending physician. Suitable dosages are in the range of 0.01-100.0 µg/kg. Wide variations in the needed dosage are to be expected in
15 view of the variety of polypeptides and fragments available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by i.v. injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization as is well understood in the art. Administrations can be single or multiple (e.g., 2- or 3-, 4-, 6-, 8-, 10-, 20-, 50-, 100-,
20 150-, or more fold). Encapsulation of the polypeptide in a suitable delivery vehicle (e.g., polymeric microparticles or implantable devices) may increase the efficiency of delivery, particularly for oral delivery.

Alternatively, a polynucleotide containing a nucleic acid sequence encoding the B7-
25 H1 polypeptide or functional fragment can be delivered to an appropriate cell of the animal. Expression of the coding sequence will preferably be directed to lymphoid tissue of the subject by, for example, delivery of the polynucleotide to the lymphoid tissue. This can be achieved by, for example, the use of a polymeric, biodegradable microparticle or microcapsule delivery vehicle, sized to optimize phagocytosis by phagocytic cells such as
30 macrophages. For example, PLGA (poly-lacto-co-glycolide) microparticles approximately 1-10 µm in diameter can be used. The polynucleotide is encapsulated in these microparticles, which are taken up by macrophages and gradually biodegraded within the cell, thereby

releasing the polynucleotide. Once released, the DNA is expressed within the cell. A second type of microparticle is intended not to be taken up directly by cells, but rather to serve primarily as a slow-release reservoir of nucleic acid that is taken up by cells only upon release from the micro-particle through biodegradation. These polymeric particles should therefore be large enough to preclude phagocytosis (i.e., larger than 5µm and preferably larger than 20µm).

Another way to achieve uptake of the nucleic acid is using liposomes, prepared by standard methods. The vectors can be incorporated alone into these delivery vehicles or co-incorporated with tissue-specific antibodies. Alternatively, one can prepare a molecular conjugate composed of a plasmid or other vector attached to poly-L-lysine by electrostatic or covalent forces. Poly-L-lysine binds to a ligand that can bind to a receptor on target cells [Cristiano et al. (1995), *J. Mol. Med.* 73, 479]. Alternatively, lymphoid tissue specific targeting can be achieved by the use of lymphoid tissue-specific transcriptional regulatory elements (TRE) such as a B lymphocyte, T lymphocyte, or dendritic cell specific TRE. Lymphoid tissue specific TRE are known [Thompson et al. (1992), *Mol. Cell. Biol.* 12, 1043-1053; Todd et al. (1993), *J. Exp. Med.* 177, 1663-1674; Penix et al. (1993), *J. Exp. Med.* 178, 1483-1496]. Delivery of "naked DNA" (i.e., without a delivery vehicle) to an intramuscular, intradermal, or subcutaneous site, is another means to achieve *in vivo* expression.

In the relevant polynucleotides (e.g., expression vectors) the nucleic acid sequence encoding the B7-H1 polypeptide or functional fragment of interest with an initiator methionine and optionally a targeting sequence is operatively linked to a promoter or enhancer-promoter combination.

Short amino acid sequences can act as signals to direct proteins to specific intracellular compartments. For example, hydrophobic signal peptides (e.g., MAISGVPVLGFFIIAVLMSAQESWA (SEQ ID NO:6)) are found at the amino terminus of proteins destined for the ER. While the sequence KFERQ (SEQ ID NO:7) (and other closely related sequences) is known to target intracellular polypeptides to lysosomes, other sequences (e.g., MDDQRDLISNNEQLP (SEQ ID NO:8) direct polypeptides to endosomes. In addition, the peptide sequence KDEL (SEQ ID NO:9) has been shown to act as a retention signal for the ER. Each of these signal peptides, or a combination thereof, can be used to traffic the B7-H1 polypeptides or functional fragments of the invention as desired.

DNAs encoding the B7-H1 polypeptides or functional fragments containing targeting signals will be generated by PCR or other standard genetic engineering or synthetic techniques.

A promoter is a TRE composed of a region of a DNA molecule, typically within 100 basepairs upstream of the point at which transcription starts. Enhancers provide expression specificity in terms of time, location, and level. Unlike a promoter, an enhancer can function when located at variable distances from the transcription site, provided a promoter is present. An enhancer can also be located downstream of the transcription initiation site. To bring a coding sequence under the control of a promoter, it is necessary to position the translation initiation site of the translational reading frame of the peptide or polypeptide between one and about fifty nucleotides downstream (3') of the promoter. The coding sequence of the expression vector is operatively linked to a transcription terminating region.

Suitable expression vectors include plasmids and viral vectors such as herpes viruses, retroviruses, vaccinia viruses, attenuated vaccinia viruses, canary pox viruses, adenoviruses and adeno-associated viruses, among others.

Polynucleotides can be administered in a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are biologically compatible vehicles which are suitable for administration to a human, e.g., physiological saline. A therapeutically effective amount is an amount of the polynucleotide which is capable of producing a medically desirable result (e.g., an enhanced T cell response) in a treated animal. As is well known in the medical arts, the dosage for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosages will vary, but a preferred dosage for administration of polynucleotide is from approximately 10^6 to 10^{12} copies of the polynucleotide molecule. This dose can be repeatedly administered, as needed. Routes of administration can be any of those listed above.

Ex Vivo Approaches

Peripheral blood mononuclear cells (PBMC) can be withdrawn from the patient or a suitable donor and exposed *ex vivo* to an activating stimulus (see above) and a B7-H1 polypeptide or polypeptide fragment (whether in soluble form or attached to a solid support by standard methodologies). The PBMC containing highly activated T cells are then introduced into the same or a different patient.

An alternative *ex vivo* strategy can involve transfecting or transducing cells obtained from the subject with a polynucleotide encoding an B7-H1 polypeptide or functional fragment-encoding nucleic acid sequences described above. The transfected or transduced cells are then returned to the subject. While such cells would preferably be hemopoietic cells (e.g., bone marrow cells, macrophages, monocytes, dendritic cells, or B cells) they could also be any of a wide range of types including, without limitation, fibroblasts, epithelial cells, endothelial cells, keratinocytes, or muscle cells in which they act as a source of the B7-H1 polypeptide or functional fragment for as long as they survive in the subject. The use of hemopoietic cells, that include the above APC, would be particular advantageous in that such cells would be expected to home to, among others, lymphoid tissue (e.g., lymph nodes or spleen) and thus the B7-H1 polypeptide or functional fragment would be produced in high concentration at the site where they exert their effect, i.e., enhancement of an immune response. In addition, if APC are used, the APC expressing the exogenous B7-H1 molecule can be the same APC that presents an alloantigen or antigenic peptide to the relevant T cell. The B7-H1 can be secreted by the APC or expressed on its surface. Prior to returning the recombinant APC to the patient, they can optionally be exposed to sources of antigens or antigenic peptides of interest, e.g., those of tumors, infectious microorganisms, or autoantigens. The same genetic constructs and trafficking sequences described for the *in vivo* approach can be used for this *ex vivo* strategy. Furthermore, tumor cells, preferably obtained from a patient, can be transfected or transformed by a vector encoding a B7-H1 polypeptide or functional fragment thereof. The tumor cells, preferably treated with an agent (e.g., ionizing irradiation) that ablates their proliferative capacity, are then returned to the patient where, due to their expression of the exogenous B7-H1 (on their cell surface or by secretion), they can stimulate enhanced tumoricidal T cell immune responses. It is understood that the tumor cells which, after transfection or transformation, are injected into the patient, can also have been originally obtained from an individual other than the patient.

The *ex vivo* methods include the steps of harvesting cells from a subject, culturing the cells, transducing them with an expression vector, and maintaining the cells under conditions suitable for expression of the B7-H1 polypeptide or functional fragment. These methods are known in the art of molecular biology. The transduction step is accomplished by any standard means used for *ex vivo* gene therapy, including calcium phosphate, lipofection, electroporation, viral infection, and biolistic gene transfer. Alternatively, liposomes or

polymeric microparticles can be used. Cells that have been successfully transduced are then selected, for example, for expression of the coding sequence or of a drug resistance gene. The cells may then be lethally irradiated (if desired) and injected or implanted into the patient.

Methods of Screening for Compounds that Inhibit or Enhance Immune Responses.

The invention provides methods for testing compounds (small molecules or macromolecules) that inhibit or enhance an immune response. Such a method can involve, e.g., culturing a B7-H1 polypeptide of the invention (or a functional fragment thereof) with T cells in the presence of a T cell stimulus (see above). Useful B7-H1 polypeptides include those with amino acid sequences identical to wild-type sequences or they can contain one or more (e.g., one, two, three, four, five, six, seven, eight, nine, 10, 12, 14, 17, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more) conservative substitutions. The B7-H1 polypeptide can be in solution or membrane bound (e.g., expressed on the surface of the T cells) and it can be natural or recombinant. Compounds that inhibit the T cell response will likely be compounds that inhibit an immune response while those that enhance the T cell response will likely be compounds that enhance an immune response.

The invention also relates to using B7-H1 or functional fragments thereof to screen for immunomodulatory compounds that can interact with B7-H1. One of skill in the art would know how to use standard molecular modeling or other techniques to identify small molecules that would bind to T cell interactive sites of B7-H1. One such example is provided in Broughton (1997) Curr. Opin. Chem. Biol. 1, 392-398.

A candidate compound whose presence requires at least 1.5-fold (e.g., 2-fold, 4-fold, 6-fold, 10-fold, 150-fold, 1000-fold, 10,000-fold, or 100,000-fold) more B7-H1 in order to achieve a defined arbitrary level of T cell activation than in the absence of the compound can be useful for inhibiting an immune response. On the other hand, a candidate compound whose presence requires at least 1.5 fold (e.g., 2-fold, 4-fold, 6-fold, 10-fold, 100-fold, 1000-fold, 10,000 fold, or 100,000-fold) less B7-H1 to achieve a defined arbitrary level of T cell activation than in the absence of the compound can be useful for enhancing an immune response. Compounds capable of interfering with or modulating B7-H1 function are good candidates for immunosuppressive immunoregulatory agents, e.g., to modulate an autoimmune response or suppress allogeneic or xenogeneic graft rejection.

B7-H1 Antibodies

The invention features antibodies that bind to either or both of the B7-H1 polypeptides or fragments of such polypeptides. Such antibodies can be polyclonal antibodies present in the serum or plasma of animals (e.g., mice, rabbits, rats, guinea pigs, sheep, horses, goats, cows, or pigs) which have been immunized with the relevant B7-H1 polypeptide or peptide fragment using methods, and optionally adjuvants, known in the art. Such polyclonal antibodies can be isolated from serum or plasma by methods known in the art. Monoclonal antibodies that bind to the above polypeptides or fragments are also embodied by the invention. Methods of making and screening monoclonal antibodies are well known in the art.

Once the desired antibody-producing hybridoma has been selected and cloned, the resultant antibody can be produced in a number of methods known in the art. For example, the hybridoma can be cultured *in vitro* in a suitable medium for a suitable length of time, followed by the recovery of the desired antibody from the supernatant. The length of time and medium are known or can be readily determined.

Additionally, recombinant antibodies specific for B7-H1, such as chimeric and humanized monoclonal antibodies comprising both human and non-human portions, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example, using methods described in Robinson et al., International Patent Publication PCT/US86/02269; Akira et al., European Patent Application 184,187; Taniguchi, European Patent Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger et al., PCT Application WO 86/01533; Cabilly et al., U.S. Patent No. 4,816,567; Cabilly et al., European Patent Application 125,023; Better et al. (1988) *Science* 240, 1041-43; Liu et al. (1987) *J. Immunol.* 139, 3521-26; Sun et al. (1987) *PNAS* 84, 214-18; Nishimura et al. (1987) *Canc. Res.* 47, 999-1005; Wood et al. (1985) *Nature* 314, 446-49; Shaw et al. (1988) *J. Natl. Cancer Inst.* 80, 1553-59; Morrison, (1985) *Science* 229, 1202-07; Oi et al. (1986) *BioTechniques* 4, 214; Winter, U.S. Patent No. 5,225,539; Jones et al. (1986) *Nature* 321, 552-25; Veroeayan et al. (1988) *Science* 239, 1534; and Beidler et al. (1988) *J. Immunol.* 141, 4053-60.

Also included within the scope of the invention are antibody fragments and derivatives which contain at least the functional portion of the antigen binding domain of an

antibody that binds specifically to B7-H1. Antibody fragments that contain the binding domain of the molecule can be generated by known techniques. For example, such fragments include, but are not limited to: F(ab')₂ fragments which can be produced by pepsin digestion of antibody molecules; Fab fragments which can be generated by reducing the disulfide bridges of F(ab')₂ fragments; and Fab fragments which can be generated by treating antibody molecules with papain and a reducing agent. See, e.g., National Institutes of Health, 1 Current Protocols In Immunology, Coligan *et al.*, ed. 2.8, 2.10 (Wiley Interscience, 1991). Antibody fragments also include Fv (e.g., single chain Fv (scFv)) fragments, *i.e.*, antibody products in which there are no constant region amino acid residues. Such fragments can be produced, for example, as described in U.S. Patent No. 4,642,334 which is incorporated herein by reference in its entirety.

The following examples are meant to illustrate, not limit, the invention.

Example 1. Materials and Methods

Cloning of hB7-H1 cDNA and construction of Ig fusion proteins. The 5' and 3' ends of hB7-H1 cDNA were amplified by PCR from a human placenta cDNA library synthesized by SMART PCR cDNA synthesis kit (Clontech, Palo Alto, CA). The primer pairs used for the PCR were derived from the placenta library plasmid and from the expressed sequence tag (EST) clone AA292201. A cDNA clone that included an orf encoding hB7-H1 of hB7-H1 cDNA was amplified by PCR from the same cDNA library by specific primers and cloned into the pcDNA3 vector (Invitrogen, Carlsbad, CA) and sequenced. The amino acid sequences of hB7-H1, B7-1 and B7-2 were analyzed using the ClustalW algorithm with BLOSUM 30 matrix (MacVector, Oxford Molecular Group). The hB7-H1Ig fusion protein was prepared by fusing the extracellular domain of hB7H-1 to the CH2-CH3 domain of mouse IgG2a in the expression plasmid pmlgV and the resulting construct was transfected into CHO cells. An analogous method was also used for preparation of B7-1Ig, CTLA4Ig and ICOSIg fusion proteins. The fusion proteins were purified from culture supernatants by passage over a Protein G –Sepharose affinity columns (Pharmacia, Uppsala, Sweden) and the purified fusion proteins were dialyzed into endotoxin-free PBS.

DNA transfection. Plasmids containing nucleic acid sequences encoding full length hB7-H1 (pcDNA3-hB7-H1), B7-1 (pCDM8-B7.1) or control parental vectors without coding sequences were transfected into 293 cells or COS cells by calcium phosphate or DEAE-

Dextran transfection (Promega, Madison, WI). After 48 hours of incubation, the expression levels of hB7-H1 or B7-1 on transfectants were determined by fluorescence flow cytometry (FFC) with an antiserum specific for hB7-H1 or anti-B7-1 monoclonal antibody (mAb) (PharMingen), respectively.

5 Mice and cell lines. Female C57BL/6 (B6), DBA/2, and BALB/c mice were purchased from the National Cancer Institute (Frederick, MD). CD28^{-/-} mice with a B6 genetic background were kindly provided by Dr. Moses Rodrigues (Department of Immunology, Mayo Clinic, Rochester, MN). P815 mastocytoma, L1210 lymphoma, EL4 mouse T-cell lymphoma and 293 human kidney epithelial cells were purchased from the American Type Culture Collection (Manassas, VA). Cell lines were maintained in a complete medium containing RPMI-1640 (Life Technologies, Rockville, MD) supplemented with 10% fetal bovine serum (FBS) (HyClone, Logan, UT), 25 mM HEPES, penicillin G (100 U/ml) and streptomycin sulfate (100 µg/ml).

15 T-cell and cytokine assays. For human T cell studies, PBMC were isolated from the blood of healthy human volunteer donors by Ficoll-Hypaque gradient centrifugation. The PBMC were passed through a nylon wool column to obtain purified T cells (~85% of CD3⁺ cells), or were subjected to further purification (>95% of CD3⁺ cells) using an anti-CD4/8 MACS magnetic bead system (Miltenyl Biotec, Germany). For co-stimulation assays, purified T cells at a concentration of 1×10^5 cells/well were cultured in triplicate in 96-well flat-bottomed microtiter tissue culture plates that were pre-coated overnight with antibody specific for human CD3 (HIT3a, PharMingen, Palo Alto, CA) and either hB7-H1Ig (5 µg/ml) or control Ig (purified mouse IgG2a or murine 4-1BBIg fusion protein). In some experiments, the microtiter wells were coated with only antibody specific for CD3 and B7-1- or hB7-H1-transfected COS cells were used (10^4 cells/well) as a source of the co-stimulatory molecules. To measure cytokine production, supernatants were collected at 24, 48 and 72 hours after initiation of the cultures and the concentrations of IL-2, IL-4, IFN-γ and IL-10 were determined by sandwich ELISA (PharMingen) according to the manufacturer's instructions. Wells containing B7-1Ig or antibody specific for human CD28 (CD28.2, PharMingen) were included for comparison or as a positive control, respectively. T cell proliferation was determined by the addition of 1.0 µCi [³H]-thymidine per well on day 2 followed by at least 18 hours of additional culture. Incorporated [³H]-thymidine was determined using a MicroBeta TriLux liquid scintillation counter (Wallac, Finland).

For mixed lymphocyte reaction (MLR) assays, purified human T cells (2×10^5 cells/well) were co-cultured in triplicate with allogeneic human PBMC (4000 Rad-irradiated) at 2×10^5 cells/well in the presence of soluble hB7-H1Ig or control Ig. Four days later, T cell proliferation was determined by [3 H]-thymidine incorporation. Neutralizing mAb specific for human IL-2 (Clone MQ1-17H12, PharMingen) was added at 8 μ g/ml in the beginning of T cell cultures. Polymyxin B (10 μ g/ml) was also included in the assays of cell proliferation and cytokine secretion to completely neutralize any contaminating endotoxin.

For mouse T cell studies, T cells were purified by passing lymph node or spleen cells through a nylon wool column. CD4⁺ or CD8⁺ T cells were positively selected by magnetic sorting using FITC-conjugated mAb against CD4 or CD8 and microbeads coated with antibody specific for fluorescein isothiocyanate (FITC) (MiltenyiBiotec, Auburn, CA) according to the manufacturer's instructions. The purity of isolated CD4⁺ and CD8⁺ T cells was > 95% by FFC with mAb specific for mouse CD4 and CD8, respectively. Purified T cells at 2×10^6 /ml from mouse spleens were cultured in 96-well plates that were pre-coated with mAb specific for mouse CD3 in the presence of mB7-H1Ig or control mouse IgG2a ("control Ig") also coated onto the culture well bottoms. mAb specific for mouse CD28 (2.5 μ g/ml) was used in soluble form as a positive control co-stimulator. Proliferation of T cells was determined by incorporation of [3 H]-thymidine (1.0 μ Ci/well) added 15 h before harvesting of the 3-day cultures. [3 H]-thymidine incorporation was determined by a MicroBeta TriLux liquid scintillation counter (Wallac, Turku, Finland). To detect cytokines, supernatants were collected between 18-72 h of culture and the concentrations of IFN- γ , IL-2, IL-10, IL-4, and GM-CSF were measured by sandwich ELISA following the manufacturer's (PharMingen) instructions.

Nucleic acid analysis. Northern blot analysis of human RNA was carried out using commercially available human multiple tissue Northern blot membranes (Clontech, Palo Alto, CA). Membranes were incubated in ExpressHyb hybridization solution (Clontech) for 30 min at 68 °C. The random-primed cDNA probe was full length hB7-H1 encoding cDNA (870 bp) labeled using [32 P]-dCTP. A 32 P-labeled human β -actin cDNA probe (2.0 kb) was used as a control. Hybridization was carried out for 1 hr at 68 °C, the membranes were washed 3 times in 2 x SSC containing 0.05% SDS, and were then exposed at -80 °C to x-ray film.

Tissue distribution of mB7-H1 mRNA was carried out using commercially available multiple tissue mouse RNA dot blot membranes (Clontech) according to the manufacturer's instructions. The random-primed cDNA probe was full-length mB7-H1 encoding cDNA and was labeled using [³²P]-dCTP. The hybridization was performed for 16h at 65°C. After washing four times with 2x SSC containing 0.05% SDS, the membranes were exposed at -80°C to x-ray films.

Antibodies and fusion proteins. Rabbit antibodies against mB7-H1 protein were prepared by Cocalico Biologicals (Reamstown, PA) by immunization of rabbits with a keyhole limpet hemocyanin (KLH)-conjugated hydrophilic peptide spanning amino acids 95-119 of mB7-H1 ("peptide 95-119") (GNAALQITDVKLQDAGVYCCIIISYG) (SEQ ID NO:16). Polyclonal antibody was purified from rabbit serum using an affinity column containing insoluble matrix material conjugated with the peptide 95-119. Both ELISA and FFC analysis of COS cells transfected with an expression vector containing cDNA encoding mB7-H1 demonstrated that the polyclonal antibody bound specifically to mB7-H1. Purified mAb specific for mouse CD3 and mouse CD28 and FITC-conjugated mAb specific for mouse CD4, mouse CD8, and mouse CD40L, phycoerythrin (PE)-conjugated mAb specific for mouse CD3, mouse B220 and mouse Mac-1 were purchased from PharMingen (San Diego, CA). FITC-conjugated goat antibody specific for rabbit IgG was purchased from Southern Biotechnology Associates (Birmingham, AL). Purified rabbit IgG and hamster IgG were purchased from Rockland (Gilbertsville, PA).

To prepare the mB7-H1Ig fusion protein, cDNA encoding the mB7-H1 extracellular domain was generated by RT-PCR using the sense primer 5'-CAGGAATTCACCATGAGGATATTTGCTG-3' (SEQ ID NO:17) and the anti-sense primer 5'-CATCAGATCTATGTGAGTCCTGTTCTGTG-3' (SEQ ID NO:18) from mouse T cell mRNA. After digestion with EcoRI and BglII, the PCR products were fused to the CH2-CH3 domain of mouse IgG2a heavy chain in the expression plasmid pmIgV [Dong et al. (1999) *Nature Med.* 5, 1365-1369]. The resulting plasmid, pmB7-H1Ig, was transfected into CHO cells. Stably transfected cells were cultured in serum-free CHO media (Life Technologies). The mB7-H1Ig in the supernatants was purified using a protein G-Sepharose column (Pierce, Rockford, IL) and dialyzed into LPS-free PBS. The endotoxin concentration was less than 1 pg/mg of purified protein according to the limulus amoebocyte lysate assays (CAPE COD, Woods Hoke, MA). The mB7-1Ig fusion protein containing the extra cellular

domain of mB7-1 fused to the Ch2-CH3 domain of mouse IgG-2a heavy chain was prepared by an analogous method.

Fluorescence flow cytometry analysis. To prepare an antiserum specific for hB7-H1, mice were immunized with purified hB7-H1Ig emulsified in complete Freund's adjuvant (Sigma) and boosted three times with hB7-H1Ig in incomplete Freund's adjuvant. Serum was collected and the specificity was determined by ELISA and by FACS staining (1:1000 dilution) of hB7-H1 cDNA-transfected 293 cells or COS cells. Pre-injection mouse serum was used as a control.

To prepare activated human T and B cells, freshly isolated human PBMC (10×10^6 cells/ml) were activated with 5 μ g/ml of PHA (Sigma) or 10 μ g/ml of LPS (Sigma), respectively. For preparation of activated monocytes, adherent PBMCs were cultured in 1,500 IU/ml of recombinant human IFN- γ (Biosource, Camarillo, CA) and 100 ng/ml of LPS. All cultures were harvested and analyzed at 48 hours. For direct immunofluorescence staining, T cells were incubated at 4 °C with 1 μ g of either fluorescein- (FITC) or phycoerythrin- (PE) conjugated mAb for 30 min and analyzed by FACScan flow cytometry (Becton Dickinson, Mountain View, CA) with Cell Quest software (Becton Dickinson) as described previously. The mAb specific for CD3 (UCHT1), CD4 (RPA-T4), CD8 (RPA-T8), CD14 (M5E2), CD19 (B43), CD28 (CD28.2), CD80 (BB1) were purchased from PharMingen. For indirect immunofluorescence staining, cells were first incubated with anti-hB7-H1 antibody (1:1000), 5 μ g of ICOSIg or CTLA4Ig at 4 °C. After 30 min, the cells were washed and further incubated with FITC- (Biosource, Camarillo, CA) or PE-conjugated (Southern Biotechnology Associates, Inc., Birmingham, AL) goat anti-human or anti-mouse IgG F(ab')₂ for 30 min at 4 °C. The human or mouse IgG1 protein (Sigma) or mouse 4-1BBIg (mouse 4-1BB extracellular domain fused with the Fc of human IgG1 or mouse IgG2a) was used as control Ig. In some experiments, Fc receptors were blocked by human or mouse Ig before incubation with FITC- or PE-conjugated mAbs.

For indirect immunofluorescence analysis of mouse cells, the cells were incubated with the antibodies at 4°C for 30 min in the presence of blocking mAb specific for CD16/32 (Fc receptor) (PharMingen). The cells were washed and further incubated with FITC-conjugated anti-rabbit IgG. The cells were then stained with PE-conjugated mAb specific for mouse CD3, mouse B220, or mouse Mac-1. Fluorescence was analyzed with a FACS Calibur flow cytometer and analyzed with Cell Quest software (Becton Dickinson, Mountain

View, CA). To prepare activated mouse T cells, nylon-wool-purified mouse T cells (>75% CD3⁺ cells) at a concentration of 2×10^6 /ml were cultured with mAb specific for mouse CD28 (5 µg/ml) and mouse CD3 (5 µg/ml). For preparation of activated mouse B cells, mouse splenocytes were cultured with LPS (10 µg/ml; Sigma, St. Louis, MO). Mouse macrophages were obtained from the peritoneal cavities of mice which had been injected with thioglycollate 7 days before. For activation, the mouse peritoneal exudate cells (PEC) were cultured with IFN-γ (10 U/ml) and LPS (100 ng/ml). All cultures were harvested and the cells analyzed at 48 h. To detect CD40L expression, CD4⁺ T cells were purified by magnetic sorting (see above), cultured as indicated, and incubated with FITC-conjugated mAb to CD40L.

Cytotoxic T-lymphocyte (CTL) generation. To generate alloantigen-specific CTL activity *in vitro*, nylon wool purified T cells (2.5×10^6 /ml) from B6 splenocytes were stimulated in 24-well tissue culture plates with irradiated (10,000 rads) mock.P815, mB7-1⁺ P815, or mB7-H1⁺ P815 cells (2.5×10^5 /ml) for 5 days. After the 5-day stimulation, CTL activities against P815 (H-2^d) and EL4 (H-2^b) were measured in a standard ⁵¹Cr release assay [Chen et al. (1994) *J. Exp. Med.* 179, 523-532; Li et al. (1996) *J. Exp. Med.* 183, 639-644].

To generate tumor-specific CTL activity *in vivo*, DBA/2 mice were inoculated subcutaneously (s.c.) with 1×10^6 mock.P815, mB7-1⁺ P815, or mB7-H1⁺ P815 cells. The draining lymph nodes were removed 7-10 d after tumor injection and the suspended lymph node cells (3×10^6 /ml) were re-stimulated in 24 well tissue culture plates with wild type irradiated (10,000 rads) P815 cells (3×10^5 /ml) for 5 days. The cells were harvested and their CTL activity was measured in a standard ⁵¹Cr release assay against wild type P815 tumor target cells.

In vivo induction and assay of TNP-specific antibody. Trinitrophenol (TNP) conjugated to KLH (TNP-KLH; 100 µg/mouse) (Biosearch Technologies, Novato, CA) in phosphate buffered saline (PBS) was injected i.p. into B6 mice on day 0. On days 1 and 4, the mice were injected i.p. with 100 µg of control Ig, mB7-1Ig, or mB7-H1Ig. Sera were collected on days 7 and 14. To measure TNP-specific antibodies in the sera, 0.3 mg/ml TNP-BSA (Biosearch Technologies) was coated onto the well-bottoms of 96-well ELISA plates overnight at 4°C. Non-specific binding sites in the ELISA plates were blocked with 10% FBS in PBS for 90 min at room temperature. After extensive washing, samples (diluted by 1/200-1/2000 with PBS) were added and incubated for 2 h. The plates were then washed and

biotinylated rat antibodies specific for mouse IgM, IgG1, IgG2a, IgG2b, or IgG3 (PharMingen) were added to the wells. The plates were further incubated for 1 h at room temperature. After washing the plates, horseradish peroxidase (HRP)-conjugated streptavidin (Caltag Laboratories, Burlingame, CA) was added to the wells and the plates were incubated for 1 h at room temperature. The plates were washed and the solutions in all wells was measured. 3,3',5,5'-tetramethyl-benzidine substrate (Sigma) was added to the wells. The OD₄₅₀ for the solutions in all wells was measured.

T cell proliferation in response to KLH. B6 mice were immunized with 100 µg TNP-KLH in IFA s.c. or in PBS i.p. on day 0 and were injected i.p. with 100 µg of either mB7-H1Ig or control Ig on days 1 and 4. To detect T cell responses to KLH, draining lymph nodes and spleens were removed from immunized mice on day 7 and 14, respectively. Suspended lymph node or spleen cells were cultured with KLH at 1.56-100 µg/ml as indicated. T cell proliferation in response to KLH was determined by addition of 1 µCi/well [³H]-thymidine 15 h before harvesting of the 3-day cultures. [³H]-thymidine incorporation was measured with a MicroBeta TriLux liquid scintillation counter (Wallac).

Example 2. Molecular cloning and expression pattern of the hB7-H1 gene

A homology search of the human cDNA EST database using published human B7-1 and B7-2 amino acid sequences revealed an EST sequence (GeneBank #AA292201) encoding a homologue to human B7-1 and B7-2 molecules. The 5'- and 3'- sequences were obtained by several independent reverse transcriptase-coupled polymerase chain reactions (RT-PCR) from a human placenta cDNA library utilizing vector and EST sequences as primers. A 3,616 bp fragment that included the hB7-H1 encoding orf was cloned and sequenced (SEQ ID NO:5) (FIG. 1). The coding sequence for hB7-H1 (SEQ ID NO:2) spans nucleotides 72-951 of SEQ ID NO:5. The amino acid sequence of full-length hB7-H1 (SEQ ID NO:1) is shown in FIG. 2a. The extracellular domain of hB7-H1 has greater homology to B7-1 (20% amino acid identity) than to B7-2 (15%) (Fig. 2b) whereas its cytoplasmic domain is highly divergent from that of B7-1 and B7-2 based on analysis using the McVector 6.5 software. The open reading frame of the gene encodes a type I transmembrane protein of 290 amino acids consisting of a 22 amino acid signal peptide, Ig V-like domain, and Ig C-like domains, a hydrophobic transmembrane domain and a cytoplasmic tail of 30 amino acids (FIG. 2a). Four structural cysteines (labeled by stars in FIG. 2b), which are apparently

involved in forming the disulfide bonds of the Ig V and Ig C domains are well conserved in all B7 members (FIG. 2b) [Fargeas, C.A. et al. (1995) *J. Exp. Med.* 182, 667-675; Bajorath, J. et al. (1994) *Protein Sci.* 3, 2148-50; Linsley, P.S. et al. (1994) *Immunity* 1, 793-801; Inaba, K. et al. (1994) *J. Exp. Med.* 180, 1849-60; Freeman, G. J. et al. (1995) *Immunity* 2, 523-532]. In addition, the tyrosine residue in B7-1 (at position 87) and in B7-2 (at position 82) of the Ig V-like domain is conserved in hB7-H1 (at position 81) (FIG. 2b).

Northern blot analysis revealed that expression of the hB7-H1 mRNA was abundant in heart, skeletal muscle, placenta and lung but was weak in thymus, spleen, kidney and liver (FIG. 3). The hB7-H1 mRNA was not detectable in brain, colon, small intestine and peripheral blood mononuclear cells (PBMC). In most of the tissues in which hB7-H1 mRNA was detectable, two transcripts of approximately 4.1 and 7.2 kb were found.

An expression plasmid containing the extracellular domain of hB7-H1 fused in frame with the Fc portion (CH2 and CH3-domains) of the mouse IgG2a was constructed. The resulting product, hB7-H1Ig fusion protein, was purified from the supernatants of CHO cells transfected with the plasmid and was used for immunization of the mice to prepare a hB7-H1-specific antiserum. Fluorescence flow cytometry analysis using the hB7-H1-specific antiserum showed that freshly isolated CD3+ T and CD19+ B cells express negligible levels of hB7-H1 while a fraction (~16%) of CD14+ monocytes constitutively express hB7-H1. hB7-H1 can, however, be up-regulated by cell activation. Approximately 30% of PHA-treated CD3+ T cells and 90% of CD14+ monocytes (treated with IFN- γ and LPS) express hB7-H1. Only 6% of CD19+ B cells after LPS activation express hB7-H1 (FIG. 4). Confirmatory results were obtained by RT-PCR analysis.

Transfection of the plasmid pcDNA3-hB7-H1 into 293 cells (B7-H1/293 cells) led to the expression of hB7-H1 as detected by hB7-H1-specific antiserum (FIG. 5a). The binding of antibody was eliminated by the inclusion of soluble hB7-H1Ig in the staining mixture (FIG. 5a, arrow), thereby demonstrating specificity of the antiserum. Neither CTLA4Ig nor ICOSIg bound to hB7-H1/293 cells. Although both CTLA4Ig and ICOSIg bound to Raji cells, the binding was not blocked by the inclusion of hB7-H1Ig (FIG. 5a, arrows). Taken together with the observation that hB7-H1Ig did not bind to Jurkat cells (FIG. 5b, right panel), despite their constitutive expression of CD28 (FIG. 5b, left panel), the above results indicate that hB7-H1 is not a ligand for CD28, CTLA-4, or ICOS.

Example 3. Co-stimulation of T cell proliferation by hB7-H1

To assess whether hB7-H1 co-stimulates T-cell growth, T cells purified (>95% purity) from PBMC of healthy human donors were stimulated with hB7-H1Ig in the presence of suboptimal doses of mAb specific for human CD3. T cell proliferation in 3-day cultures was determined by incorporation of [³H]-thymidine. hB7-H1Ig, immobilized on culture plates, enhanced T cell proliferation up to 10-fold compared to the control Ig in the presence of 5-20 ng/ml of mAb specific for human CD3, also immobilized on the culture plates. In the absence of mAb specific for human CD3, hB7-H1Ig at a concentration up to 5 µg/ml induced no T cell proliferation (FIG. 6a). If hB7-H1Ig was included in the cultures without immobilization, its co-stimulatory effect was significantly decreased. Consistent with this observation, the inclusion of soluble hB7-H1Ig at levels of 0.6-5 µg/ml in allogeneic MLR moderately (~2-fold) increased the proliferation of T cells (FIG. 6b). Thus, hB7-H1 can promote and co-stimulate proliferative responses of T cells to polyclonal T cell stimuli and to allogeneic antigens.

Example 4. hB7-H1 co-stimulation preferentially induces the production of IL-10
and the co-stimulatory effect requires IL-2

The levels of IL-2, IL-4, IFN-γ, and IL-10 produced by T cells after co-stimulation with hB7-H1Ig, B7-1Ig, or mAb specific for human CD28 in the presence of mAb specific for human CD3 (FIG. 7a-7d) were measured. Similar to B7-1Ig and anti-CD28, immobilized hB7-H1Ig antibody dramatically increased the production of IL-10 by T cells in response to immobilized mAb specific for human CD3 after stimulation for 48 and 72 hours (FIG. 7a). IL-10 was not detected if T cells were co-stimulated with immobilized control Ig. The level of IFN-γ was also significantly elevated by co-stimulation with immobilized hB7-H1Ig (FIG. 7b). In contrast to B7-1Ig and mAb specific for human CD28, hB7-H1Ig co-stimulated low or negligible levels of IL-2 (FIG. 7c) and IL-4 (FIG. 7d), respectively. These observations were reproducible in six independent experiments. These results show that co-stimulation by hB7-H1 preferentially stimulates the production of IL-10.

The production of IL-2, although low, peaked at 24 hours upon hB7-H1 co-stimulation (FIG. 7c), while IL-10 secretion started to increase only after 48 and 72 hours (FIG. 7a). Increasing concentrations of hB7-H1Ig led to a small increase (< 1 ng/ml) of IL-2 secretion (FIG. 7e). To determine the roles of the early-produced IL-2, the effects of mAb

specific for human IL-2 on T cell proliferation and IL-10 production in B7H-mediated co-stimulation were tested. Similar to T cell proliferation induced by B7-1-COS cells and immobilized mAb specific for human CD3, T cell proliferation induced by hB7-H1-COS cells and mAb specific for human CD3 was blocked by inclusion of mAb specific for human IL-2 (FIG. 8a). Furthermore, IL-10 secretion from hB7-H1Ig-co-stimulated T cells was also inhibited by mAb specific for human IL-2 (FIG. 8b). Therefore, the hB7-H1 co-stimulation of both T cell growth and IL-10 secretion is an IL-2-dependent process.

Example 5. hB7-H1 co-stimulation increases apoptosis of activated T cells.

To determine the effect of hB7-H1 ligation on the viability of activated T cells, the proportion of live T cells remaining after activation with an optimally activating dose of mAb specific for human CD3 in the presence of immobilized hB7-H1Ig was determined by trypan blue staining. A consistent decrease of alive T cells was observed. At the end of culture, T cells were stained with annexin V and propidium iodide (PI) to distinguish the early phase and late phase of apoptosis, respectively. The apoptotic cells in early phase (annexin V-positive, PI-negative) were significantly increased to 24.8 % in the presence of hB7-H1Ig compared to 14.2 % in the absence of hB7-H1Ig in 5 experiments ($P = 0.001$). A representative experiment is shown in FIG. 9a (upper panel). Similar results were obtained using hB7-H1Ig-treated Jurkat cells (control Ig: 38.3% vs. hB7-H1Ig: 54.6%) (FIG. 9a, lower panel). The increased apoptosis was associated with upregulation of Fas and FasL expression on hB7-H1 co-stimulated T cells (FIG. 9b). These results indicated that hB7-H1 co-stimulation increased activation-induced T cell apoptosis moderately, and the increased apoptosis was associated with elevated expression of Fas and FasL.

Example 6. Production of monoclonal antibodies specific for hB7-H1.

Using standard protocols, BALB/c mice were immunized with purified hB7-H1Ig and splenocytes from the immunized mice were fused with X63-AG8.653 mouse myeloma cells. Five hybridoma lines were found to secrete antibodies specific for hB7-H1 in that, as detected by fluorescence flow cytometry, culture supernatants from these hybridoma lines positively stained hB7-H1/293 cells but did not stain control vector/293 cells. Furthermore, some of the antibodies inhibited the co-stimulatory activity of hB7-H1.

Example 7. Molecular Cloning and expression pattern of a mouseB7-H1 (mB7-H1) gene

Starting with two overlapping mouse EST clones (AA823166 and AA896104), and using a strategy similar to that for the hB7-H1 gene, a cDNA fragment that included an orf encoding mB7-H1 was cloned. The coding sequence for mB7-H1 (SEQ ID NO:4) (FIG. 10) was obtained and the amino acid sequence of mB7-H1 (SEQ ID NO: 3) (FIG. 11) was derived from it. The length of mB7-H1 is identical to that of hB7-H1 and it has the same conserved cysteine residues found in hB7-H1 (see Example 2). A cDNA fragment encoding full-length mB7-H1 was cloned into the pcDNA3 vector (Invitrogen, Carlsbad, CA) to give mB7-H1.pcDNA3.

mB7-H1, like hB7-H1, is a type I transmembrane protein of 290 amino acids that has 69% overall amino acid homology to hB7-H1 (FIG. 12a). Similar to other members of B7 family, mB7-H1 consists of an Ig V-like domain, an Ig C-like domain, a hydrophobic transmembrane domain and a cytoplasmic tail. mB7-H1 shares 20% homology to mouse B7-1, 14% to mouse B7-2, and 19% to mouse B7h/B7RP-1, based on analysis using McVector 6.5 software (Clustal W Program) (FIG. 12b).

RNA analysis revealed that mB7-H1 mRNA is abundant in mouse heart, spleen, lung, skeletal muscle and liver, and less abundant but present in mouse kidney, liver, thymus, and thyroid. Thus, the expression pattern of mB7-H1 mRNA is similar to that of human B7-H1 mRNA. Negligible expression of the mB7-H1 mRNA was observed in pancreas and testis.

FFC analysis using the anti-mB7-H1 antibody showed that resting mouse CD3⁺ T cells do not express mB7-H1 (FIG. 13, upper panel). However, a small fraction of B220⁺ mouse B cells and Mac-1⁺ mouse macrophages expressed a low level of mB7-H1 (FIG. 13, upper panel). Stimulation of mouse T cells with antibodies specific for mouse CD3 and mouse CD28 moderately increased the mB7-H1 expression on T cells. Activation of mouse B cells with LPS and macrophages with LPS plus IFN- γ significantly increased the expression of mB7-H1 on their surfaces (FIG.13, lower panel). Thus, mB7-H1, like hB7-H1, is an inducible cell surface molecule.

Example 8. Co-stimulation of mouse T cell proliferation by mb7-H1.

To investigate the costimulatory effect of mB7-H1, nylon-wool purified mouse T cells were activated with a suboptimal dose of mAb specific for mouse CD3 (coated onto

culture well bottoms at a concentration of 200 ng/ml) and co-stimulated with various concentrations of mB7-H1Ig. mB7-H1Ig enhanced T cell proliferation by up to 5-fold compared to control Ig (FIG. 14a). The costimulatory effect of the mB7-H1Ig was dose-dependent and dependent on the presence of mAb specific for mouse CD3 since in the absence of mAb specific for mouse CD3, mB7-H1Ig (up to a concentration of mB7-H1Ig 10 µg/ml) failed to stimulate the proliferation of T cells. When nylon-wool purified mouse T cells were cultured with 293 cells transfected with either mB7-H1.pcDNA3 or control vector in the presence of suboptimal doses of mAb specific for mouse CD3, mB7-H1-transfected 293 cells also enhanced T cell proliferation substantially compared to the T cell proliferation in the presence of the control vector-transfected 293 cells. Thus, similar to hB7-H1, mB7-H1 costimulates T cell proliferation.

The role of CD28 in mB7-H1 costimulation was evaluated by comparing the effects of mB7-H1Ig costimulation on T cells isolated from CD28^{-/-} mice and from normal mice. Nylon wool purified mouse T cells were activated with two suboptimal doses of antibody specific for mouse CD3 (coated onto the well bottoms of 96 well tissue culture plates at a concentration of 0.125 µg/ml or 0.25 µg/ml) and either soluble antibody specific for mouse CD28 (2.5 µg/ml) or mB7-H1Ig or control Ig (both coated onto the well bottoms of 96 well tissue culture plates at a concentration of 10 µg/ml). As shown in FIG. 14b, while there was no co-stimulatory effect of anti-CD28 mAb on CD28^{-/-} T cells, mB7-H1Ig, induced the proliferation of both CD28^{-/-} (FIG. 14b, right panel) and CD28^{+/+} ("wt"; FIG. 14b, left panel) T cells to a similar degree. Therefore, mB7-H1 can costimulate T cell growth in a CD28-independent fashion.

In order to test whether mB7-H1 preferentially co-stimulates CD4⁺ or CD8⁺ T cells, purified CD4⁺ and CD8⁺ T cells were stimulated with mB7-H1Ig (same concentration as in the experiment shown in FIG. 14b) and mAb specific for mouse CD3 (coated onto the well bottoms of 96 well tissue culture plates at a concentration of 200 ng/ml). Proliferation of CD4⁺ T cells was enhanced about 10 fold by mB7-H1Ig and the proliferation of CD8⁺ T cells was only enhanced 2-3 fold mB7-H1Ig (FIG. 14c). Thus, the co-stimulatory effect of mB7-H1 is more potent on CD4⁺ T cells than on CD8⁺ cells.

Example 9. Co-stimulation of cytokine production by mB7-H1.

The levels of IL-10, IFN- γ , IL-2, IL-4 and GM-CSF produced by T cells activated with mAb specific for mouse CD3 and co-stimulated with either mB7-H1Ig or anti-CD28 mAb were measured. FIG. 15a shows that mB7-H1Ig, similar to mAb specific for mouse CD28, co-stimulates the production of high levels of IL-10 in the day 3 cultures. IL-10 was not detectable at day 3 when T cells were treated with either control Ig and mAb specific for mouse CD3 or mAb specific for mouse CD3 alone. mB7-H1 and mAb specific for mouse CD28 enhanced the production of IFN- γ and GM-CSF. In contrast to mAb specific for mouse CD28, which induced high levels of IL-2 and IL-4, mB7-H1Ig induced no or negligible levels of IL-2 and IL-4 at all time points (FIG. 15a). Thus, mB7-H1 and hB7-H1 co-stimulate the production of a similar spectrum of cytokines.

Since IL-2 was undetectable in culture supernatants from mB7-H1Ig co-stimulated cultures, it seemed possible that mB7-H1 ligation inhibited IL-2 secretion. To test this possibility, the effect of mB7-H1 on IL-2 secretion by T cells activated by mAb specific for mouse CD3 and co-stimulated with mAb specific for mouse CD28 was tested. FIG. 15b shows that inclusion of immobilized mB7-H1Ig (at concentrations up to 10 μ g/ml) in the culture resulted in a small decrease in IL-2 production during the 18-48 h culture period that was statistically insignificant; in several repeat experiments no statistically significant decrease was ever seen. Similarly, mB7-H1Ig did not inhibit IL-2 production in cultures in which T cells were activated by mAb specific for mouse CD3 alone (FIG. 15b). The results thus indicate that mB7-H1 ligation does not inhibit the production of IL-2.

Example 10. Expression of mB7-H1 on transfected P815 tumor cells and decreased growth rate of the transfected P815 cells in mice.

Mouse (DBA/2) P815 mastocytoma cells were stably transfected with the expression plasmid (mB7-H1.pcdNA3) containing the coding sequence for mB7-H1 using FUGENETM (Roche, Mannheim, Germany) according to the manufacturer's instructions. The transfected cells were selected in complete medium containing G418 (1 mg/ml; Life Technologies) and were subsequently cloned by limiting dilution. mB7-H1 expressing P815 cells were identified by FFC using the above-described anti-mB7-H1 polyclonal antibody preparation. A representative clone (mB7-H1⁺ P815) was selected for further studies. P815 clones transfected with the pc DNA vector (mock.P815) or MB7-1 (mB7-1⁺ P815) were generated

similarly [Chen et al. (1994) *J. Exp. Med.* 179, 523-532]. Using a PE-conjugated rat polyclonal antibody specific for mB7-H1 ("anti-mB7H/PE"), mB7-H1 expression was detected by FFC on the mB7H-H1⁺ P815 cells (FIG. 18b) but not on either mock transfected P815 cells ("mock.P815") (FIG. 16b) or P815 cells transfected with a construct encoding murine B7-1 ("mB7-1⁺ P815") (FIG. 17b). On the other hand, the mB7-1⁺ P815 cells were stained with a FITC-conjugated mAb specific for murine B7-1 ("anti-mB7-1-FITC") (FIG. 17a). Furthermore, inclusion of the mB7-H1 peptide used to make the polyclonal anti-mB7-H1 antibody in the staining reaction mixture completely blocked binding of the polyclonal anti-mB7-H1 antibody to the mB7-H1⁺ P815 cells.

Groups (5 mice per group) of DBA/2 mice were injected subcutaneously (s.c.) with either 2x10⁵ mock.P815 or mB7-H1⁺ P815 cells. The growth rate of the mock.P815 cells was significantly greater in 4 out of 5 injected mice (FIG. 19a) than in the 5 mice injected with mB7-H1⁺ P815 (FIG 19b). These findings indicate that the mB7-H1⁺ P815 cells were significantly more immunogenic than mock.P815 cells and, therefore, that expression of mB7-H1 expression by P815 cells enhances their ability to elicit protective immunity.

Example 11. mB7-H1 costimulation fails to enhance allogeneic and syngeneic CTL responses

To examine the effect of mB7-H1 on the generation of allogeneic CTL *in vitro*, nylon wool-purified T cells from B6 mice (H-2^b) were co-cultured with irradiated mock.P815 (H-2^d), mB7-1⁺ P815, or mB7-H1⁺ P815 cells for 5 days and the CTL activity of cells harvested from the cultures was tested against wild-type P815 target cells in standard ⁵¹Cr release assays at the indicated effector to target cell ratios ("E/T ratio"). As depicted in FIG. 20a, mB7-H1⁺ P815 cells and mock.P815 cells were poor stimulators of CTL activity. In contrast, mB7-1⁺ P815 cells elicited strong P815-specific CTL activity. The CTL induced by mB7-1 were alloantigen-specific since they did not lyse responder (B6) H-2 haplotype (H-2^b) EL4 tumor target cells. Thus, mB7-H1 expression does not facilitate the generation of allogeneic CTL.

The ability of mB7-H1⁺ P815 cells to stimulate P815 tumor specific CTL *in vivo* was tested. DBA/2 mice were injected s.c. with mock.P815, mB7-1⁺ P815, or mB7-H1⁺ P815 cells. Tumor-draining lymph nodes were removed 7 days later and T cells isolated from them were cultured with wild-type P815 cells for 5 days. Cells harvested from the cultures

were tested for CTL activity against wild-type P815 target cells in a standard ^{51}Cr release assay at the indicated E/T ratios (FIG. 20b). Effector cells from mice injected with mB7-H1⁺ P815 showed slightly increased CTL activity against P815 cells compared to effector cells from the mock.P815-injected mice; this difference in CTL activity was however statistically insignificant. In contrast, mB7-1⁺ P815 cells elicited strong CTL activity. CTL activity was P815 tumor-specific since syngeneic L1210 tumor target cells were not lysed. Thus, expression of mB7-H1 in P815 cells does not enhance the induction of CTL activity against P815 tumor antigens.

Example 12. B7-H1 costimulation amplifies antigen-specific T helper cell responses, T cell-dependent humoral responses, and expression of CD40L on T cells

To investigate the effect of mB7-H1 costimulation on T helper cell function, B6 mice were immunized with TNP-conjugated KLH on day 0, and were injected with mB7-H1Ig at day 1 and day 4. The *in vitro* proliferative responses of T cells obtained from both lymph nodes and spleens of the immunized mice to various concentrations of KLH were measured. As shown in FIG. 21, T cells from both spleens and lymph nodes of TNP-KLH-immunized mice proliferated in response to KLH in a dose dependent fashion. Administration of mB7-H1Ig to TNP-KLH-immunized mice amplified the subsequent *in vitro* proliferative responses of T cells by up to 2-3 fold. These results indicate that mB7-H1 co-stimulation enhances T helper cell responses *in vivo*.

The effect of mB7-H1 co-stimulation on the generation of antigen-specific antibodies to TNP was investigated in a system well recognized as measuring helper T cell-dependent antibody responses [Marrack and Kappler (1975) *J. Immunol.* 114, 1116-1125; Romano et al. (1975) *Proc. Natl. Acad. Sci. USA* 72, 4555-4558]. Thus, the level of antibodies specific for TNP in the sera of mice immunized with TNP-KLH was measured after treatment with control Ig, mB7-1Ig, or mB7-H1Ig. In preliminary experiments, a significant increase in the total anti-TNP IgG level was observed in sera of mice immunized with TNP-KLH and treated with mB7-H1Ig compared to sera of mice immunized with TNP-KLH and treated with control mIg. The relative levels of IgM and individual IgG subclass (IgG1, IgG2a, IgG2b and IgG3) anti-TNP antibodies elicited by TNP-KLH immunization and various co-stimulations were each measured. As shown in FIG. 22, the amount of TNP-specific IgG2a antibody was increased significantly in the sera of mice immunized with TNP-KLH and

treated with mB7-H1Ig. The effect was different from that elicited by mB7-1Ig in which the levels of antibodies specific for TNP of other IgG subclasses (IgG1 and IgG2b) were also significantly increased (FIG. 22). Thus, mB7-H1 costimulation enhances T helper cell proliferation and T helper-dependent antibody responses, particularly IgG2a responses.

5 The CD40-CD40 ligand (CD40L) interaction in T helper cell-B cell interactions is critical for the generation of antibody responses and for Ig class switching [Calderhead et al. (2000) *Curr. Top. Microbiol. Immunol.* 245, 73-99]. The effect of co-stimulation with mB7-H1Ig on the level of CD40L on T cells was investigated. Purified CD4⁺ T cells from B6 mice were stimulated with a suboptimal concentration of mAb specific for mouse CD3 in the
10 presence of mB7-H1Ig or mAb specific for mouse CD28. Expression of CD40L on T cells was detected with a mAb specific for mouse CD40L by FFC. mB7-H1Ig co-stimulation upregulated CD40L rapidly (25.3% after 4 h incubation) compared to co-stimulation with control IgG (6.6%) or antibody specific for mouse CD28 (10.5%) (FIG. 23a). The level of CD40L was also higher after 24 h on T cells co-stimulated with mB7-H1Ig than on T cells
15 co-stimulated with the either control Ig or mB7-1Ig (FIG. 23a). Similar results were obtained with using an optimal dose of mAb specific for mouse CD3 for activation (FIG. 23b). Thus, triggering of the B7-H1 counter-receptor on T cells rapidly upregulates the expression of CD40L.

20 Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

What is claimed is:

1 1. An isolated DNA comprising:

2 (a) a nucleic acid sequence that encodes a polypeptide with the ability to co-
3 stimulate a T cell, wherein the nucleic acid sequence hybridizes under stringent conditions to
4 the complement of a sequence that encodes a polypeptide with an amino acid sequence with
5 SEQ ID NO:1 or SEQ ID NO:3; or

6 (b) the complement of the nucleic acid sequence.

1 2. The DNA of claim 1, wherein the nucleic acid sequence encodes a
2 polypeptide comprising an amino acid sequence with SEQ ID NO:1.

1 3. The DNA of claim 1, wherein the nucleic acid sequence encodes a
2 polypeptide comprising an amino acid sequence with SEQ ID NO:3.

1 4. The DNA of claim 1, wherein the nucleic acid sequence has a sequence of
2 SEQ ID NO:2.

1 5. The DNA of claim 1, wherein the nucleic acid sequence has a sequence of
2 SEQ ID NO:4.

1 6. An isolated polypeptide encoded by the DNA of claim 1.

1 7. The isolated polypeptide of claim 6, wherein the polypeptide comprises an
2 amino acid sequence of amino acid residue 23 to amino acid residue 290 of SEQ ID NO:1, or
3 said amino acid sequence but differing solely by conservative substitutions.

1 8. The isolated polypeptide of claim 6, wherein the polypeptide comprises an
2 amino acid sequence of amino acid residue 23 to amino acid residue 290 of SEQ ID NO:3, or
3 said amino acid sequence but differing solely by conservative substitutions.

1 9. The isolated polypeptide of claim 6, wherein the polypeptide comprises an
2 amino acid sequence of SEQ ID NO:1, or said amino acid sequence but differing solely by
3 conservative substitutions.

10. The isolated polypeptide of claim 6, wherein the polypeptide comprises an amino acid sequence of SEQ ID NO:3, or said amino acid sequence but differing solely by conservative substitutions.

11. A vector comprising the DNA of claim 1.

12. The vector of claim 11, wherein the nucleic acid sequence is operably linked to a regulatory element which allows expression of said nucleic acid sequence in a cell.

13. A cell comprising the vector of claim 11.

14. A method of co-stimulating a T cell, the method comprising contacting the T cell with the polypeptide of claim 6.

15. The method of claim 14, wherein the contacting comprises culturing the polypeptide with the T cell *in vitro*.

16. The method of claim 14, wherein the T cell is in a mammal.

17. The method of claim 16, wherein the contacting comprises administering the polypeptide to the mammal.

18. The method of claim 16, wherein the contacting comprises administering a nucleic acid encoding the polypeptide to the mammal.

19. The method of claim 16, comprising:

(a) providing a recombinant cell which is the progeny of a cell obtained from the mammal and has been transfected or transformed *ex vivo* with a nucleic acid encoding the polypeptide so that the cell expresses the polypeptide; and

(b) administering the cell to the mammal.

20. The method of claim 19, wherein the cell is an antigen presenting cell (APC) and the cell expresses the polypeptide on its surface.

21. The method of claim 20, wherein, prior to the administering, the APC is pulsed with an antigen or an antigenic peptide.

1 22. The method of claim 16, wherein the mammal is suspected of having an
2 immunodeficiency disease.

1 23. The method of claim 16, wherein the mammal is suspected of having an
2 inflammatory condition.

1 24. The method of claim 16, wherein the mammal is suspected of having an
2 autoimmune disease.

1 25. A method of identifying a compound that inhibits an immune response, the
2 method comprising:

3 (a) providing a test compound;

4 (b) culturing, together, the compound, the polypeptide of claim 6, a T cell, and a
5 T cell activating stimulus; and

6 (c) determining whether the test compound inhibits the response of the T cell to
7 the stimulus, as an indication that the test compound inhibits an immune response.

1 26. The method of claim 25, wherein the stimulus is an antibody that binds to a T
2 cell receptor or a CD3 polypeptide.

1 27. The method of claim 25, wherein the stimulus is an alloantigen or an antigenic
2 peptide bound to a major histocompatibility complex (MHC) molecule on the surface of an
3 antigen presenting cell (APC).

1 28. The method of claim 27, wherein the APC is transfected or transformed with a
2 nucleic acid encoding the polypeptide and the polypeptide is expressed on the surface of the
3 APC.

1 29. A method of identifying a compound that enhances an immune response, the
2 method comprising:

3 (a) providing a test compound;

4 (b) culturing, together, the compound, the polypeptide of claim 6, a T cell, and a
5 T cell activating stimulus; and

6 (c) determining whether the test compound enhances the response of the T cell to
7 the antigen, as an indication that the test compound enhances an immune response.

1 30. The method of claim 29, wherein the stimulus is an antibody that binds to a T
2 cell receptor or a CD3 polypeptide.

1 31. The method of claim 29, wherein the stimulus is an alloantigen or an antigenic
2 peptide bound to a MHC molecule on the surface of an APC.

1 32. The method of claim 31, wherein the APC is transfected or transformed with a
2 nucleic acid encoding the polypeptide and the polypeptide is expressed on the surface of the
3 APC.

1 33. An antibody that binds specifically to the polypeptide of claim 6.

1 34. The antibody of claim 33, wherein the antibody is a monoclonal antibody.

1 35. The antibody of claim 33, wherein the antibody binds to the polypeptide with
2 SEQ ID NO:1.

1 36. A cell comprising the vector of claim 12.

1 37. A method of producing a polypeptide that co-stimulates a T cell, the method
2 comprising culturing the cell of claim 36 and purifying the polypeptide from the culture.

1 38. A fusion protein comprising a first domain joined to at least one additional
2 domain, wherein the first domain comprises a polypeptide of claim 6.

1 39. The fusion protein of claim 38, wherein the at least one additional domain
2 comprises the constant region of an immunoglobulin heavy chain or a fragment thereof.

1 40. A nucleic acid molecule encoding the fusion protein of claim 39.

1 41. A vector comprising the nucleic acid molecule of claim 40.

1 42. The vector of claim 41, wherein the nucleic acid molecule is operably linked
2 to a regulatory element which allows expression of the nucleic acid molecule in a cell.

1 43. A cell comprising the vector of claim 42.

1 44. A method of producing a fusion protein, the method comprising culturing the
2 cell of claim 43 and purifying the fusion protein from the culture.

1 45. The method of claim 14, wherein, the T cell is a helper T cell.

1 46. The method of claim 45, wherein the helper T cell is a helper T cell that
2 provides helper activity for a B cell antibody-producing response.

1 47. The method of claim 45, wherein the B cell antibody response is an IgG2a
2 antibody response.

1 48. The method of claim 14, wherein the co-stimulation causes an increase in the
2 level of CD40 ligand on the T cell surface.

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ABSTRACT OF THE INVENTION

The invention provides novel polypeptides useful for co-stimulating T cells, isolated nucleic acid molecules encoding them, vectors containing the nucleic acid molecules, and cells containing the vectors. Also included are methods of making and using these co-stimulatory polypeptides.

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FIG. 1

CCCACGCGTCCGCAGCTTCCCGAGGCTCCGCACCAGCCGCGCTTCTGTCCGCC
 TGCAGGGCATTCCAGAAAGATGAGGATATTTGCTGTCTTTATATTCATGACCT
 ACTGGCATTGCTGAACGCATTTACTGTCACGGTTCCCAAGGACCTATATGTG
 GTAGAGTATGGTAGCAATATGACAATTGAATGCAAATCCCAGTAGAAAAAC
 AATTAGACCTGGCTGCACTAATTGTCTATTGGGAAATGGAGGATAAGAACAT
 TATTCAATTTGTGCATGGAGAGGAAGACCTGAAGGTTTCAGCATAGTAGCTAC
 AGACAGAGGGGCCCGGCTGTTGAAGGACCAGCTCTCCCTGGGAAATGCTGCAC
 TTCAGATCACAGATGTGAAATTGCAGGATGCAGGGGTGTACCGCTGCATGAT
 CAGCTATGGTGGTGCCGACTACAAGCGAATTACTGTGAAAGTCAATGCCCA
 TACAACAAAATCAACCAAAGAATTTTGGTGTGGATCCAGTCACCTCTGAAC
 ATGAACTGACATGTCAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGAC
 AAGCAGTGACCATCAAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAG
 AGAGAGGAGAAGCTTTTCAATGTGACCAGCACACTGAGAATCAACACAACA
 ACTAATGAGATTTTCTACTGCACTTTTAGGAGATTAGATCCTGAGGAAAACCA
 TACAGCTGAATTGGTCATCCCAGAACTACCTCTGGCACATCCTCCAAATGAA
 AGGACTCACTTGGTAATTCTGGGAGCCATCTTATTATGCCTTGGTGTAGCACT
 GACATTCATCTTCCGTTTAAGAAAAGGGAGAATGATGGATGTGAAAAAATGT
 GGCATCCAAGATACAACTCAAAGAAGCAAAGTGATACACATTTGGAGGAG
 ACGTAATCCAGCATTGGAACCTTCTGATCTTCAAGCAGGGATTCTCAACCTGTG
 GTTTAGGGGTTTCATCGGGGCTGAGCGTGACAAGAGGAAGGAATGGACCCGTG
 GGATGCAGGCAATGTGGGACTTAAAAGGCCCAAGCACTGAAAATGGAACCT
 GGCGAAAGCAGAGGAGGAGAATGAAGAAAGATGGAGTCAAACAGGGAGCC
 TGGAGGGAGACCTTGATACTTTCAAATGCCTGAGGGGCTCATCGACGCCTGT
 GACAGGGAGAAAGGATACTTCTGAACAAGGAGCCTCCAAGCAAATCATCCAT
 TGCTCATCCTAGGAAGACGGGTTGAGAATCCCTAATTTGAGGGTCAGTTCCTG
 CAGAAGTGCCCTTTGCCTCCACTCAATGCCTCAATTTCTTTTCTGCATGACTG
 AGAGTCTCAGTGTTGGAACGGGACAGTATTTATGTATGAGTTTTCTCTATTTA
 TTTTGAGTCTGTGAGGTCTTCTTGTGCATGTGAGTGTGGTTGTGAATGATTTCTT
 TTGAAGATATATTGTAGTAGATGTTACAATTTTGTGCGCCAACTAACTTGCT
 GCTTAATGATTTGCTCACATCTAGTAAAACATGGAGTATTTGTAAGGTGCTTG
 GTCTCCTCTATACTACAAGTATACATTGGAAGCATAAAGATCAAACCGTTG
 GTTGCATAGGATGTCACCTTTATTTAACCCTTAATACTCTGGTTGACCTAAT
 CTTATTCTCAGACCTCAAGTGTCTGTGCAGTATCTGTTCCATTTAAATATCAG
 CTTTACAATTATGTGGTAGCCTACACACATAATCTCATTTTCATCGCTGTAACC
 ACCCTGTTGTGATAACCACTATTATTTTACCCATCGTACAGCTGAGGAAGCAA
 ACAGATTAAGTAACTTGCCCAAACCAGTAAATAGCAGACCTCAGACTGCCAC
 CCACTGTCCTTTTATAATAACAATTTACAGCTATATTTTACTTTAAGCAATTCTT
 TTATTCAAAAACCATTTATTAAGTGCCCTTGCAATATCAATCGCTGTGCCAGG
 CATTGAATCTACAGATGTGAGCAAGACAAAGTACCTGTCCTCAAGGAGCTCA
 TAGTATAATGAGGAGATTAACAAGAAAATGTATTATTACAATTTAGTCCAGT
 GTCATAGCATAAGGATGATGCGAGGGGAAAACCCGAGCAGTGTTGCCAAGA
 GGAGGAAATAGGCCAATGTGGTCTGGGACGGTTGGATATACTTAAACATCTT
 AATAATCAGAGTAATTTTCAATTTACAAAGAGAGGTCGGTACTTAAATAACC
 CTGAAAAATAACACTGGAATTCCTTTTCTAGCATTATATTTATTCCTGATTTGC

FIG. 1 (continued)

CTTTGCCATATAATCTAATGCTTGTTTATATAGTGTCTGGTATTGTTTAAACAGT
TCTGTCTTTTCTATTTAAATGCCACTAAATTTTAAATTCATACCTTTCCATGAT
TCAAAATTCAAAAGATCCCATGGGAGATGGTTGGAAAATCTCCACTTCATCC
TCCAAGCCATTCAAGTTTCCTTTCCAGAAGCAACTGCTACTGCCTTTTCATTCA
TATGTTCTTCTAAAGATAGTCTACATTTGGAAATGTATGTTAAAAGCACGTAT
TTTTAAAATTTTTTTTCTAAATAGTAACACATTGTATGTCTGCTGTGTACTTTG
CTATTTTTATTTATTTTAGTGTTCCTTATATAGCAGATGGAATGAATTTGAAGT
TCCCAGGGCTGAGGATCCATGCCTTCTTTGTTTCTAAGTTATCTTTCCCATAGC
TTTTATTATCTTTTCATATGATCCAGTATATGTTAAATATGTCCTACATATACA
TTTAGACAACCACCATTGTGTTAAGTATTTGCTCTAGGACAGAGTTTGGATTG
TTTATGTTTGCTCAAAAGGAGACCCATGGGCTCTCCAGGGTGCCTGAGTCA
ATCTAGTCCTAAAAAGCAATCTTATTATTAAGTCTGTATGACAGAATCATGTC
TGGAACCTTTGTTTTCTGCTTTCTGTCAAGTATAAACTTCACTTTGATGCTGTA
CTTGCAAAATCACATTTTCTTTCTGGAAATTCCGGCAGTGTACCTTGACTGCT
AGCTACCCTGTGCCAGAAAAGCCTCATTGCTTGTGCTTGAACCCTTGAATGCC
ACCAGCTGTCATCACTACACAGCCCTCCTAAGAGGCTTCTGGAGGTTTCGA
GATTCAGATGCCCTGGGAGATCCCAGAGTTTCCTTTCCCTCTTGGCCATATTC
TGGTGTCAATGACAAGGAGTACCTTGGCTTTGCCACATGTCAAGGCTGAAGA
AACAGTGTCTCCAACAGAGCTCCTTGTGTTATCTGTTTGTACATGTGCATTTG
TACAGTAATTGGTGTGACAGTGTTCCTTTGTGTGAATTACAGGCAAGAATTGTG
GCTGAGCAAGGCACATAGTCTACTCAGTCTATTCCTAAGTCCTAACTCCTCCT
TGTGGTGTGGATTGTGAAGGCACTTTATCCCTTTTGTCTCATGTTTCATCGTA
AATGGCATAGGCAGAGATGATACCTAATTCTGCATTTGATTGTCACTTTTTGT
ACCTGCATTAATTTAATAAAAATATTCTTATTTATTTTGTACTTGGTAAAAAA
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Fig. 2a

Signal peptide

1 MRIFAVFIFMTYWILLNAFTVTVPKDLVVEYGSNMTECKFPVEKQLDL

Ig-V-like

51 AALIVYWEMEDKNIIQFVHGEECLKVQHSSYRQRARLLKDQLSLGNAALQ

101 ITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVDPVTSE

Ig-C-like

151 HELTCQAEGYPKAEVIWTSSDHQVLSGKTTTNSKREEKLFNVTSTLRIN

201 TTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLC

TM

251 LGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET

Fig. 2b

B7-H1 30 V E Y G S N M I E K E P E K Q L D A I I E M
B7-1 43 K E V S G H N - V E E Q T R I K
B7-2 30 A Y F N T D P Q Q A N Q N Q S S E E V F E D

60 D E N I I Q F H G E E D - L K V Q S R Q A R L L
68 K M T M M S D M N - - - I V P E K N I D
60 Q E N L E N E Y L G K E X F D S V E K K M G S S

88 E D Q L S L G A A Q T D V K L Q A V E R M S Y
95 I T - - - N L S I V L A R E S E T E V V L K
90 S - - - - D S W T R L H N Q I K K L Q I H H

119 G G A Y K R - - - I T X V N A P Y K I N Q R L V V
121 Y E K A F K E L A E T K D P T S S S D P
115 K K P T G M I I Q M N S E L S Q E V P I

145 D P V E E H E L - - - Q A E K A - E V I T S S
151 E I P E I R R - I I T S G F E E - H L E E N
145 S N I E E V Y I N L E S I H E K K M V L R

171 D H Q - - - V S K T T N S K R E K F N T I T
179 G E - - - N A N V P P T R I A K I
175 T K N S T I Y D M Q K - R O O N V L L D I S

198 E I - - - T E I Y T I F R R L D P E E H T A E L
206 D F - - - M H S M L I K Y G H I V - - Q T
204 R V S F P D V S M T I F I L E T D E T L L S - S P

226 V E P E L P I A H P P N E R T
230 N W N T T K Q E F N L L
233 S E L E D P Q P H I P

FIG. 3

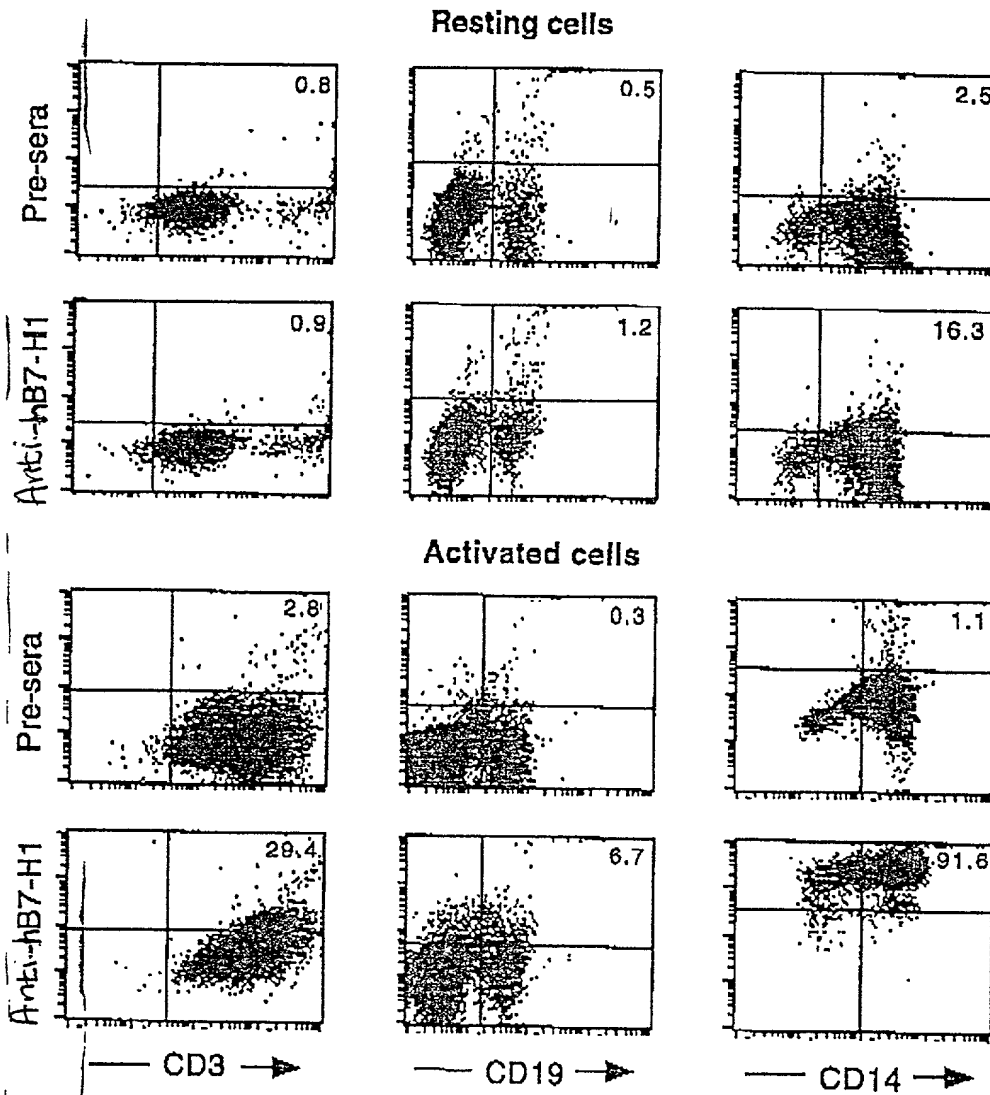
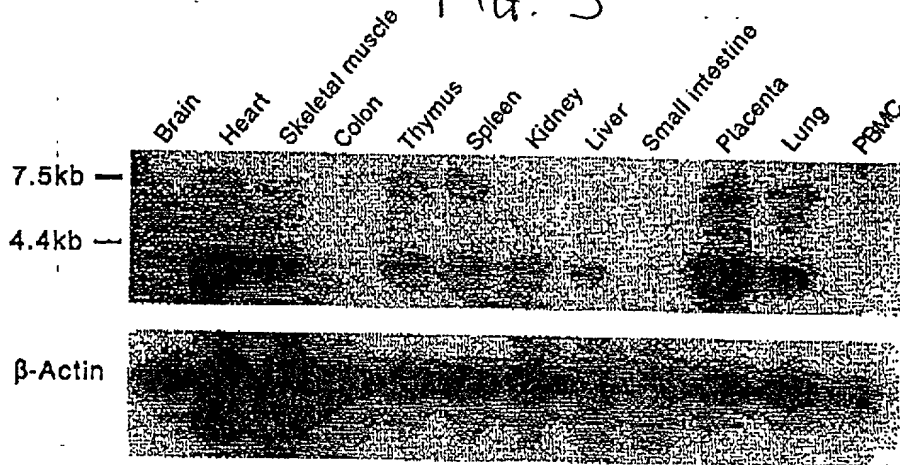


FIG. 4

FIG. 5a

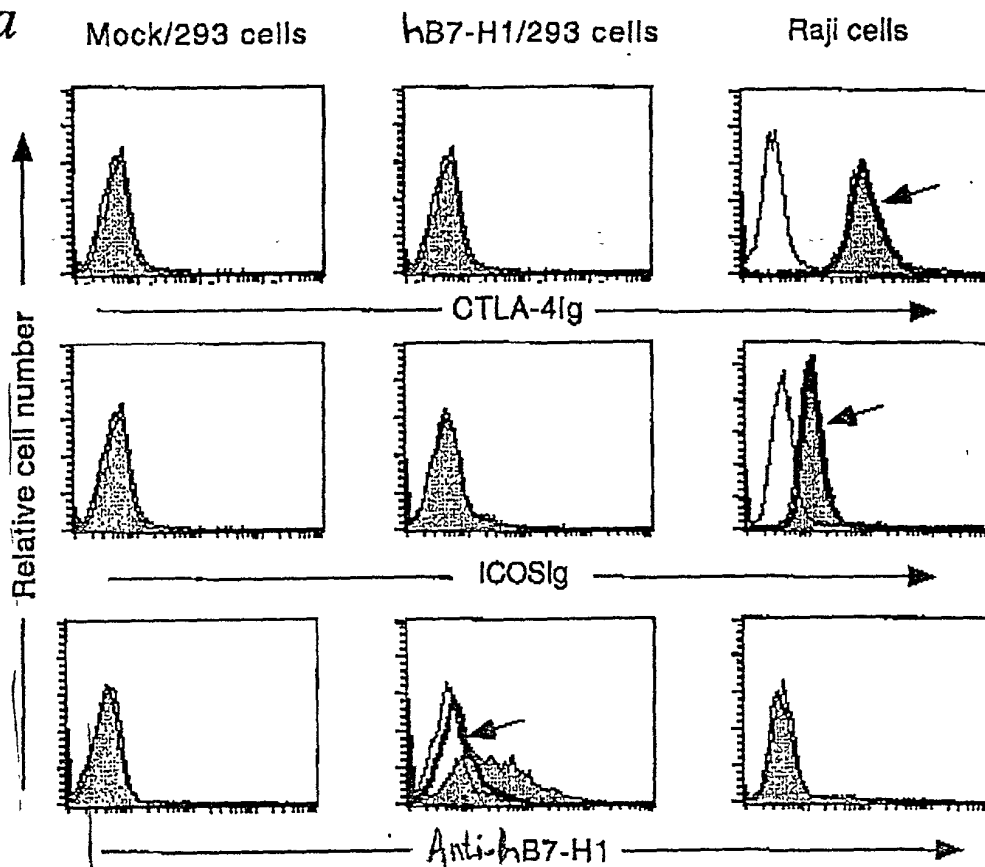


FIG. 5b

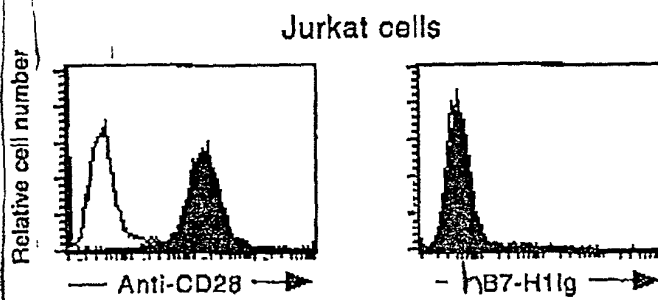


FIG. 6a

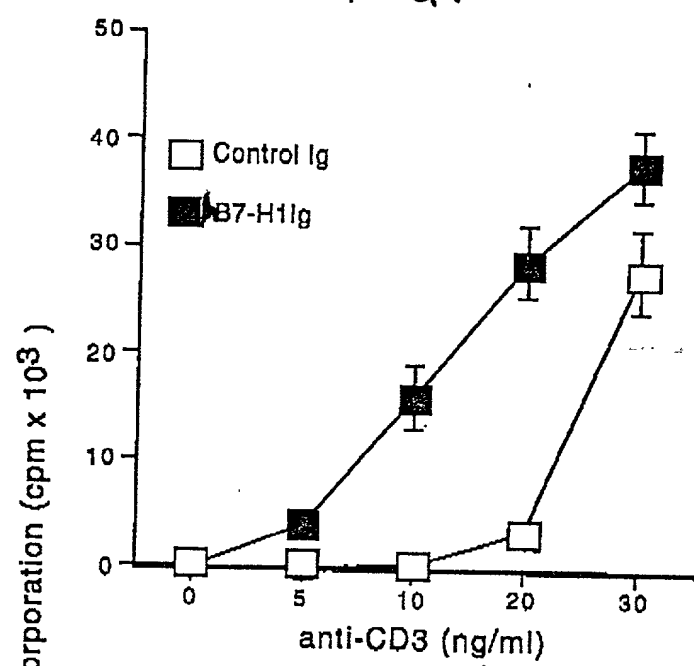
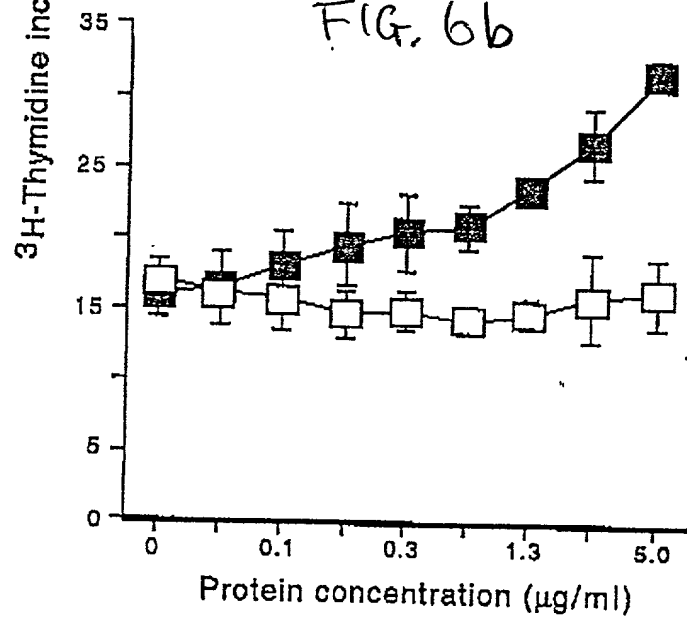


FIG. 6b



IL-10

FIG. 7a

IFN- γ

FIG. 7b

Cytokine secretion (ng/ml)

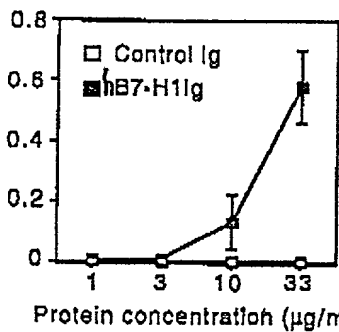
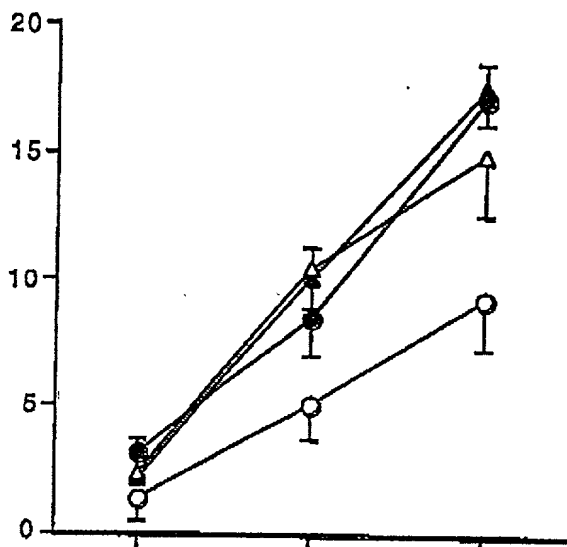
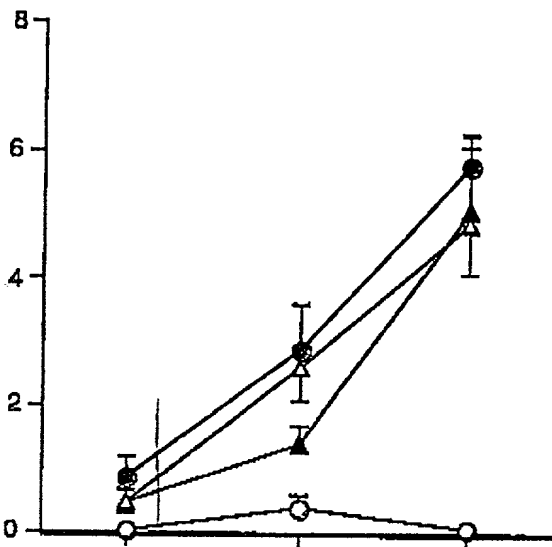


FIG. 7e

IL-2

IL-4

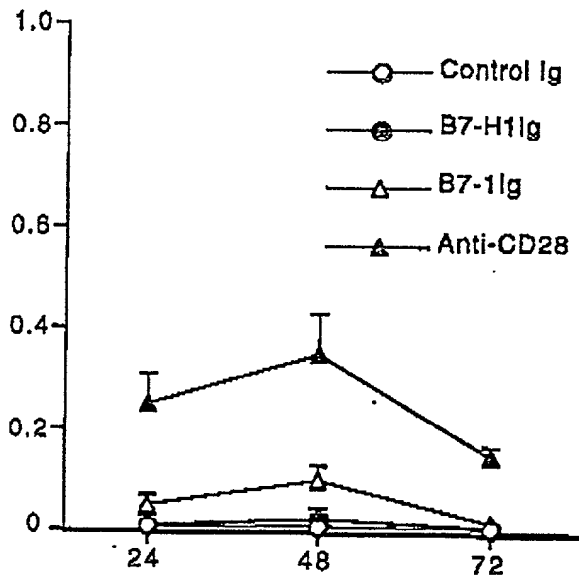
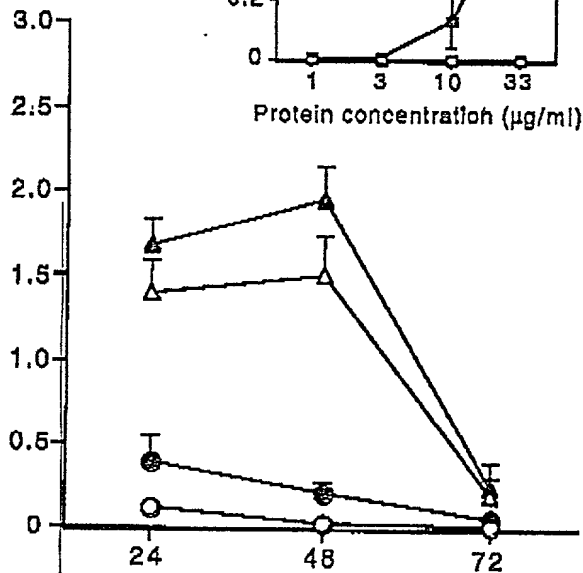


FIG. 7c

Culture time (Hours)

FIG. 7d

FIG. 8a

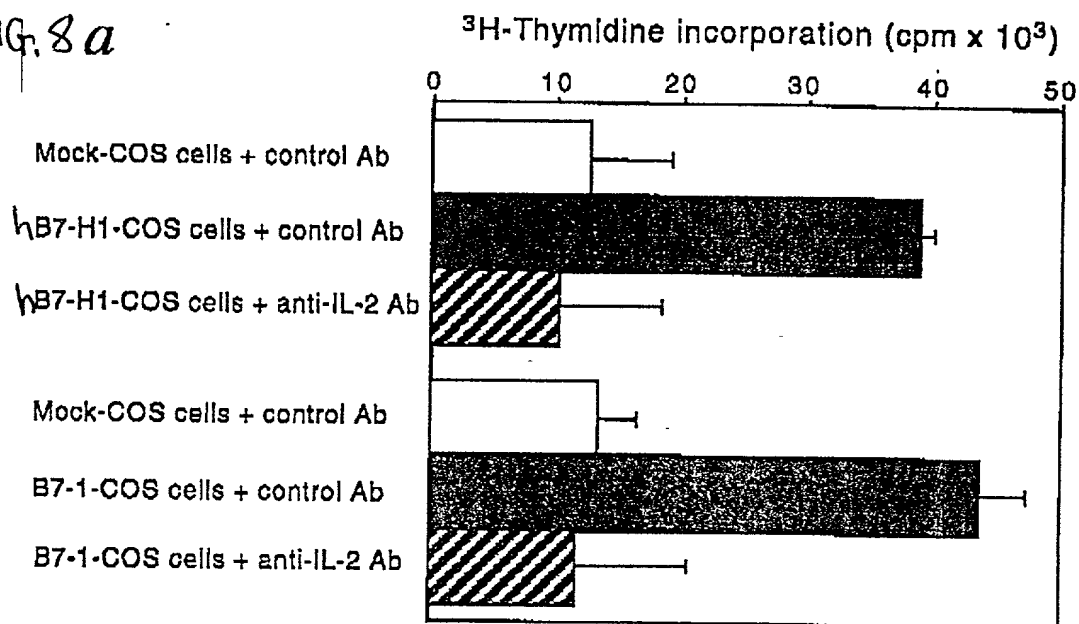


FIG. 8b

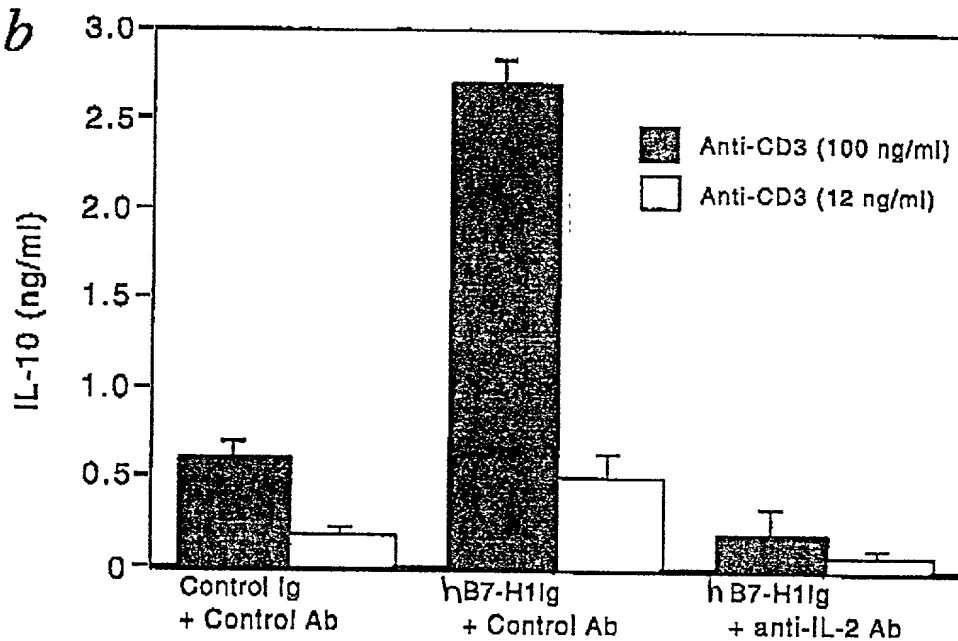


FIG. 9 a

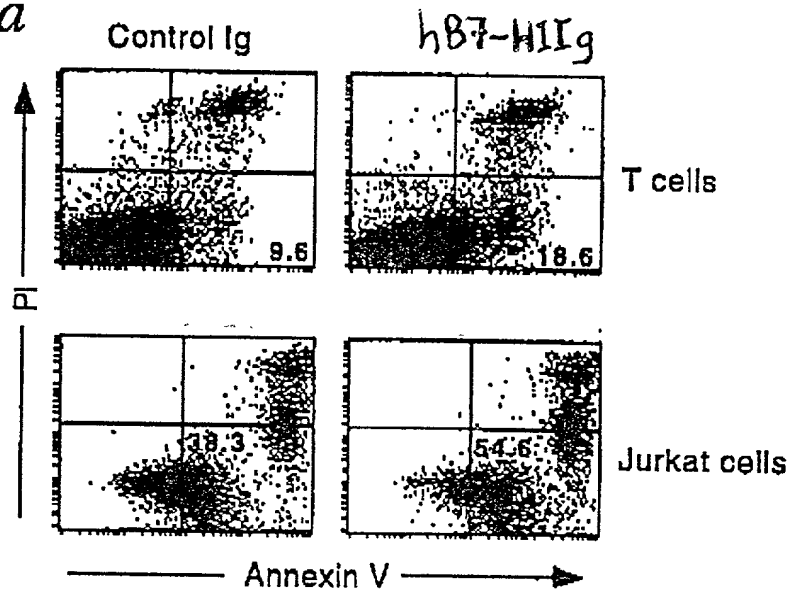


FIG. 9 b

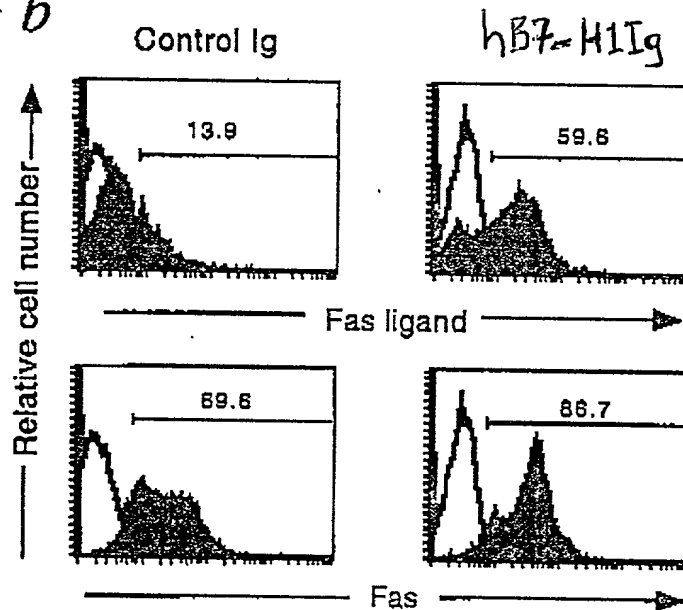


FIG. 10

ATGAGGATATTTGCTGGCATTATATTCACAGCCTGCTGTCACTTGCTACGGGC
GTTTACTATCACGGCTCCAAAGGACTTGTACGTGGTGGAGTATGGCAGCAAC
GTCACGATGGAGTGCAGATTCCCTGTAGAACGGGAGCTGGACCTGCTTGCGT
TAGTGGTGTACTGGGAAAAGGAAGATGAGCAAGTGATTTCAGTTTGTGGCAGG
AGAGGAGGACCTTAAGCCTCAGCACAGCAACTTCAGGGGGAGAGCCTCGCT
GCCAAAGGACCAGCTTTTGAAGGGAAATGCTGCCCTTCAGATCACAGACGTC
AAGCTGCAGGACGCAGGCGTTTACTGCTGCATAATCAGCTACGGTGGTGCGG
ACTACAAGCGAATCACGCTGAAAGTCAATGCCCCATACCGCAAAATCAACCA
GAGAATTTCCGTGGATCCAGCCACTTCTGAGCATGAACTAATATGTCAGGCC
GAGGGTTATCCAGAAGCTGAGGTAATCTGGACAAACAGTGACCACCAACCCG
TGAGTGGGAAGAGAAGTGTCACCACTTCCCGGACAGAGGGGATGCTTCTCAA
TGTGACCAGCAGTCTGAGGGTCAACGCCACAGCGAATGATGTTTTCTACTGT
ACGTTTTTGGAGATCACAGCCAGGGCAAAACCAACACAGCGGAGCTGATCATCC
CAGAACTGCCTGCAACACATCCTCCACAGAACAGGACTCACTGGGTGCTTCT
GGGATCCATCCTGTTGTTCCCTCATTGTAGTGTCCACGGTCCTCCTCTTCTTGAG
AAAACAAGTGAGAATGCTAGATGTGGAGAAATGTGGCGTTGAAGATACAAG
CTCAAAAAACCGAAATGATACACAATTCGAGGAGACGTAA

FIG. 11

MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVV
YWEKEDEQVIQFVAGEEDLKPQHSNFRGRASLPKDQLKGNAAALQITDVKLQDA
GVYCCIIISYGGADYKRITLKVNPYRKINQRISVDPATSEHELICQAEGYPEAEVI
WTNSDHQPVS GKRSVTTSRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQN
HTAELIPELPATHPPQNRTHWVLLGSILLFLIVVSTVLLFLRKQVRMLDVEKCGV
EDTSSKNRNDTQFEET

mouse B7-H1	1	signal peptide																							35													
		M	R	I	F	A	G	I	L	F	T	A	C	C	H	L	L	E	R	A	F	T	I	T		A	P	K	D	L	Y	V	V	E	Y	G	S	N
human B7-H1	1	M	R	I	F	A	V	F	I	E	M	T	Y	W	H	L	L	E	N	A	F	T	V	T	V	V	P	K	D	L	Y	V	V	E	Y	G	S	N
mouse B7-H1	36	IgV-like domain																							70													
		V	T	M	E	C	R	F	P	V	E	R	E	L	D	L	L	A	L	V	V	Y	W	E		K	E	D	E	Q	V	I	Q	F	V	A	G	
human B7-H1	36	M	T	I	E	C	K	F	P	V	E	K	Q	L	D	L	A	A	L	I	V	Y	W	E	M	E	D	K	N	I	I	Q	F	V	H	G		
mouse B7-H1	71	IgC-like domain																							105													
		E	E	D	L	K	P	Q	H	S	N	F	R	G	R	A	S	L	P	K	D	Q	L	L		K	G	N	A	A	L	Q	I	T	D	V	K	
human B7-H1	71	E	E	D	L	K	V	Q	H	S	S	Y	R	Q	R	A	R	L	L	K	D	Q	L	S	L	G	N	A	A	L	Q	I	T	D	V	K		
mouse B7-H1	106	IgC-like domain																							140													
		L	Q	D	A	G	V	Y	C	I	I	S	Y	G	G	A	D	Y	K	R	I	T	L	K		V	N	A	P	Y	R	K	I	N	Q	R		
human B7-H1	106	L	Q	D	A	G	V	Y	R	C	M	I	S	Y	G	G	A	D	Y	K	R	I	T	V	K	V	N	A	P	Y	N	K	I	N	Q	R		
mouse B7-H1	141	IgC-like domain																							174													
		I	L	S	-	V	D	P	A	T	S	E	H	E	L	I	C	Q	A	E	G	Y	P	E		A	E	V	I	W	T	N	S	D	H	Q	P	V
human B7-H1	141	I	L	V	V	D	P	V	T	S	E	H	E	L	T	C	Q	A	E	G	Y	P	K	A	E	V	I	W	T	S	S	D	H	Q	V	L		
mouse B7-H1	175	IgC-like domain																							209													
		S	G	K	R	S	V	T	T	S	R	T	E	G	M	L	N	V	T	S	S	L	R	V		N	A	T	A	N	D	V	F	Y	C	T		
human B7-H1	176	S	G	K	T	T	T	N	S	K	R	E	E	K	L	F	N	V	T	S	T	L	R	I	N	T	T	N	E	I	F	Y	C	T				
mouse B7-H1	210	IgC-like domain																							244													
		F	W	R	S	Q	P	G	Q	N	H	T	A	E	L	I	I	P	E	L	P	A	T	H		P	P	Q	N	R	T	E	W	V	L	L	G	
human B7-H1	211	F	R	R	L	D	P	E	E	N	H	T	A	E	L	V	I	P	E	L	P	L	A	H	P	P	N	E	R	T	H	L	V	I	L	G		
mouse B7-H1	245	TM																							279													
		S	I	L	L	F	L	I	V	V	S	T	V	L	L	F	L	R	K	Q	V	R	M	L		D	V	E	K	C	G	V	E	D	T	S		
human B7-H1	246	A	I	L	L	C	L	I	G	V	A	L	T	F	I	F	R	T	R	K	G	-	R	M	M	D	V	K	K	C	G	I	Q	D	T	N		
mouse B7-H1	280	cytoplasmic																							290													
		K	N	R	N	D	T	Q	F	E	E	T	290																									
human B7-H1	280	K	K	Q	S	D	T	H	L	E	E	T	290																									

FIG. 12a

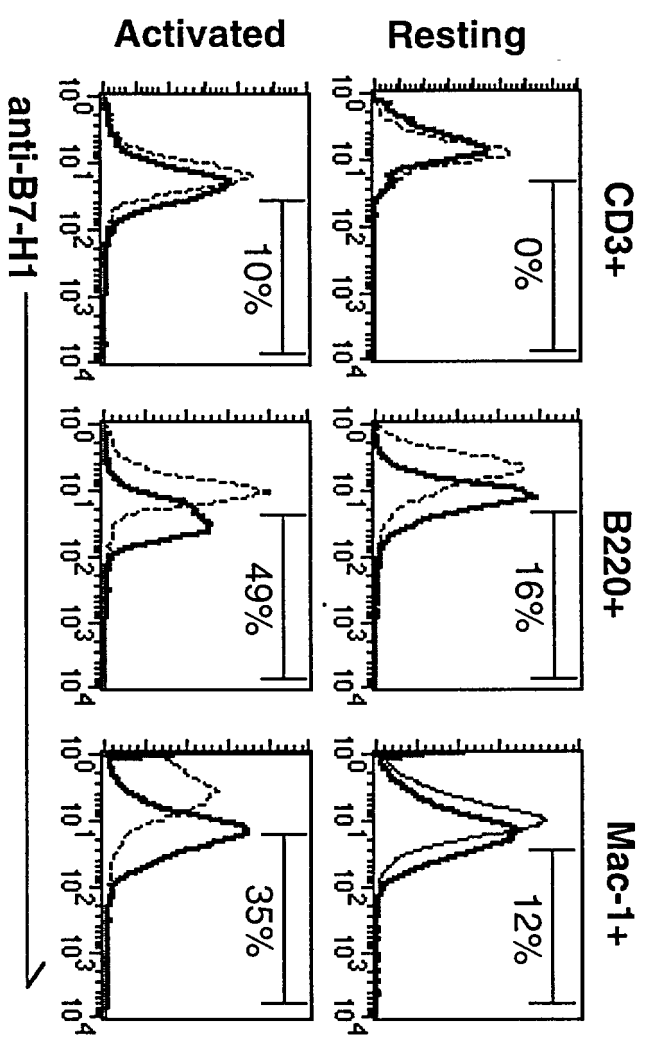


FIG. 13

FIG. 14a

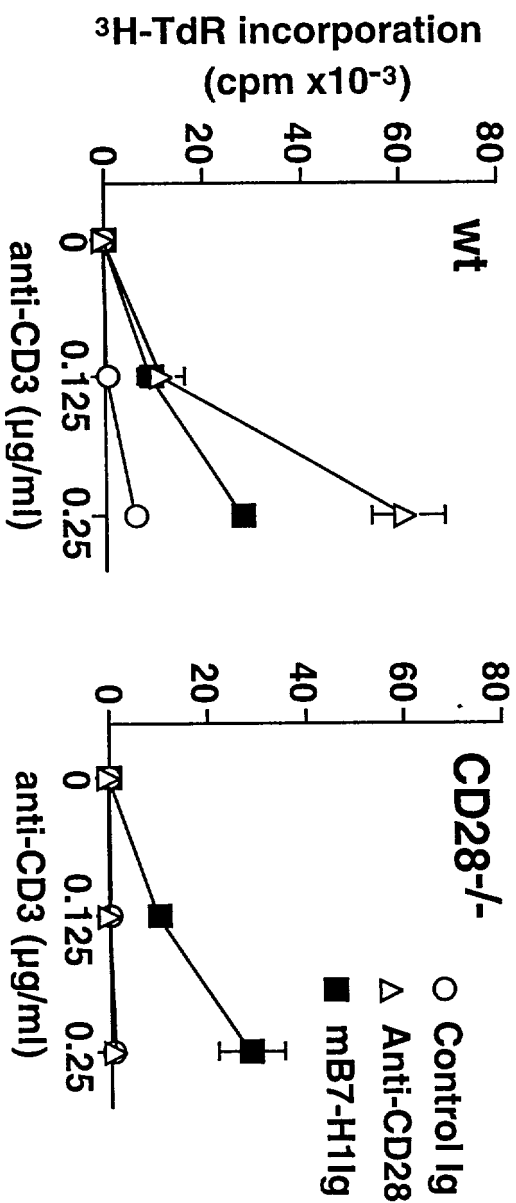
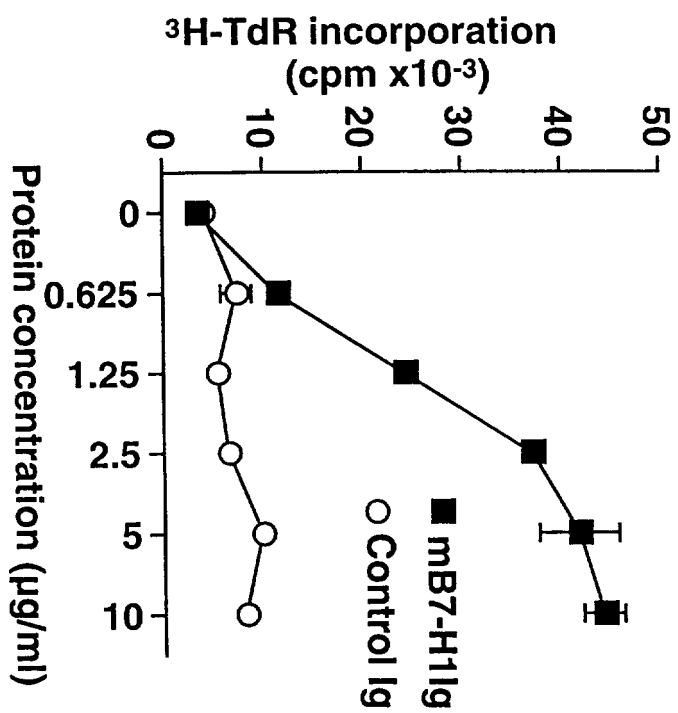


FIG. 14b

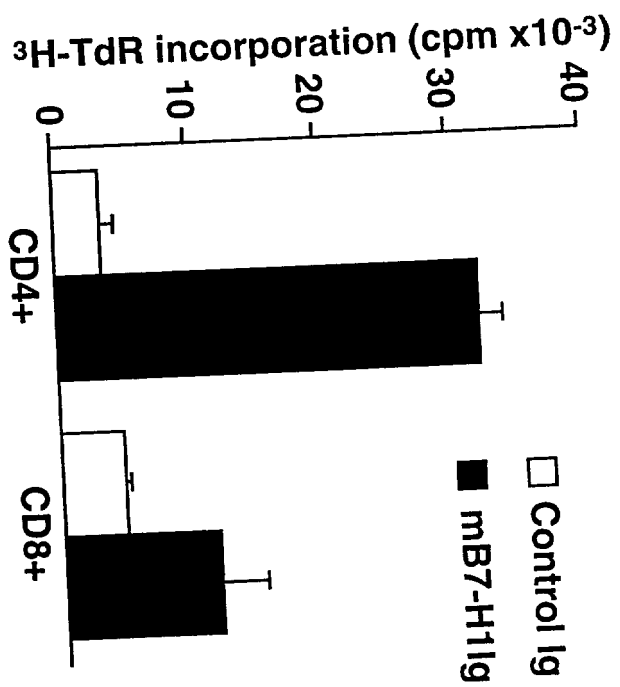


FIG. 14c

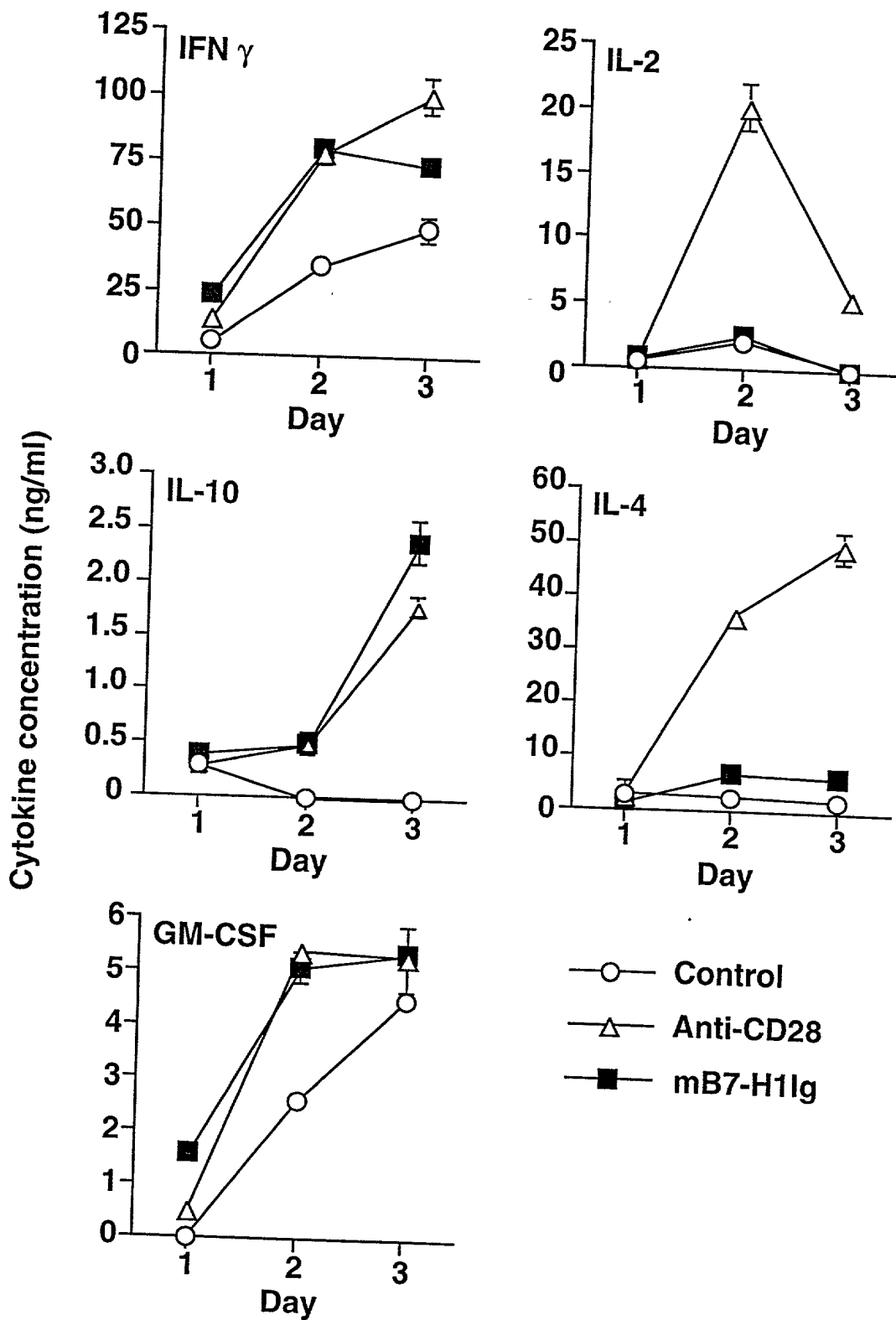


FIG. 15a

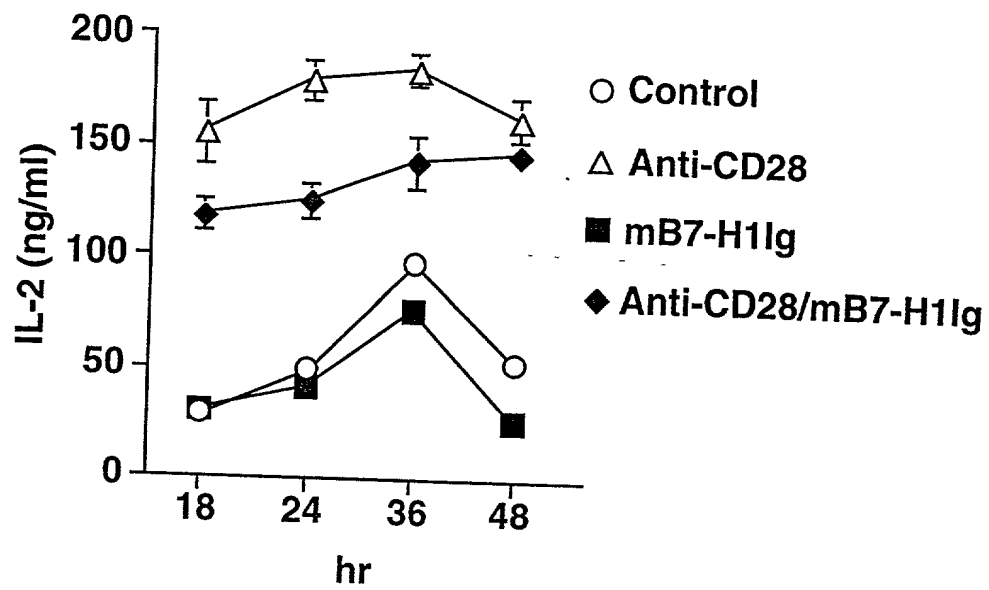


FIG. 15b

mock.P815

FIG. 16a

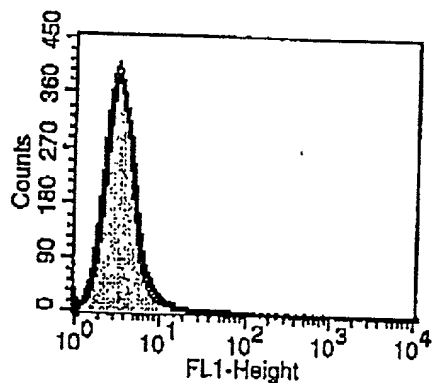
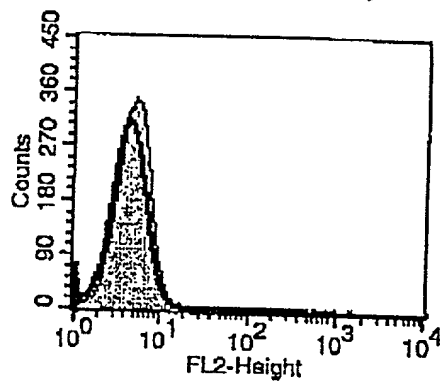


FIG. 16b



mB7-1⁺P815

FIG. 17a

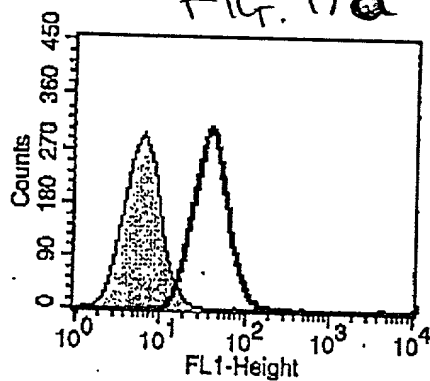
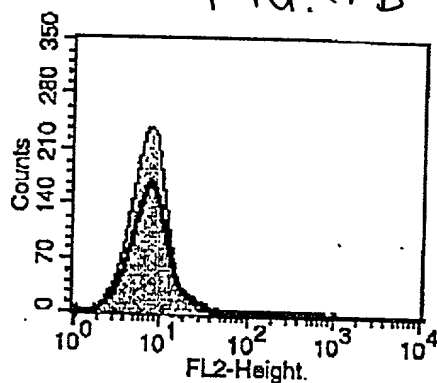


FIG. 17b



mB7-H1⁺P815

FIG. 18a

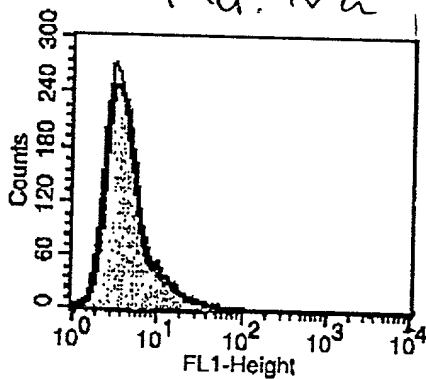
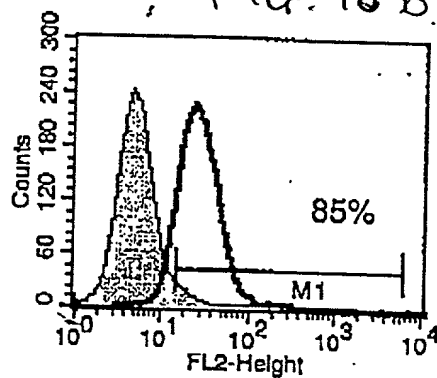


FIG. 18b



anti-mB7-1-FITC →

anti-mB7H/PE →

FIG. 19a

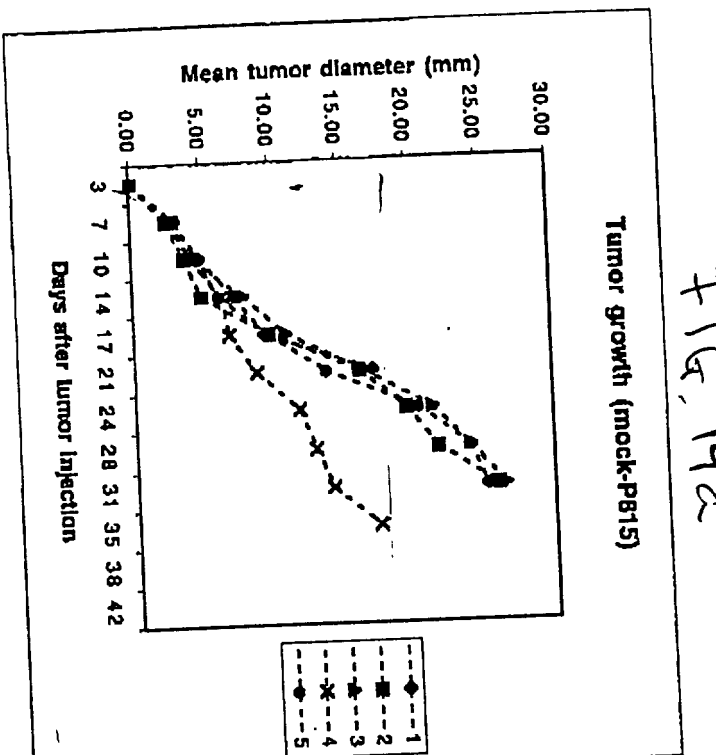


FIG. 19b

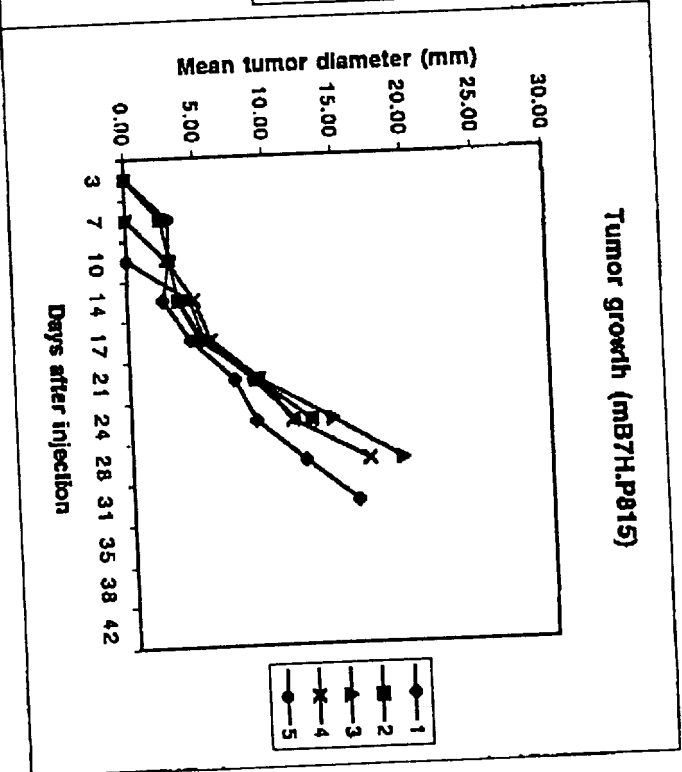


FIG. 20a

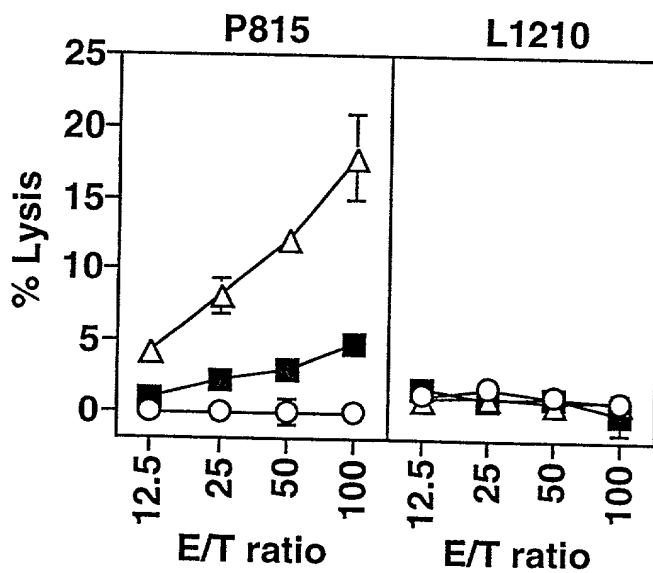
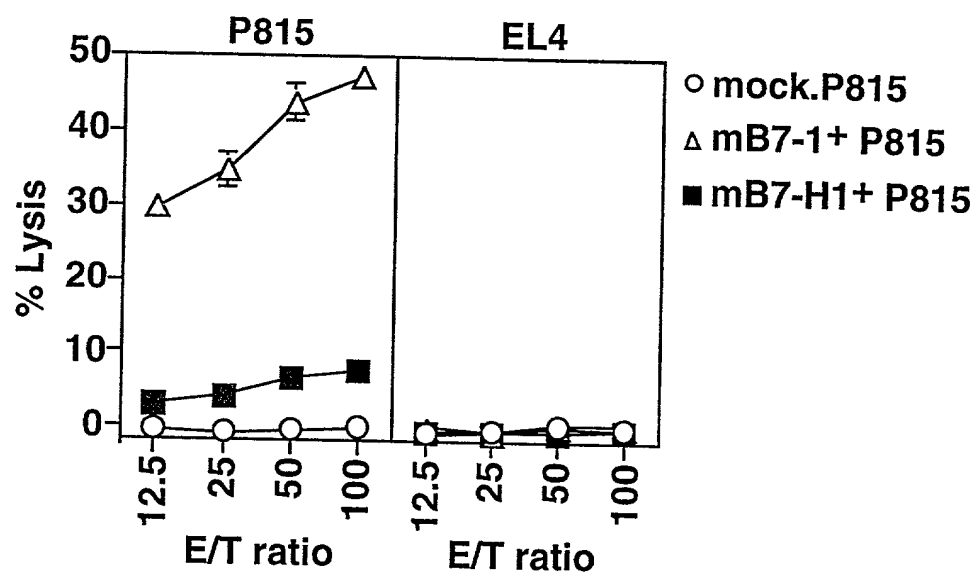


FIG. 20b

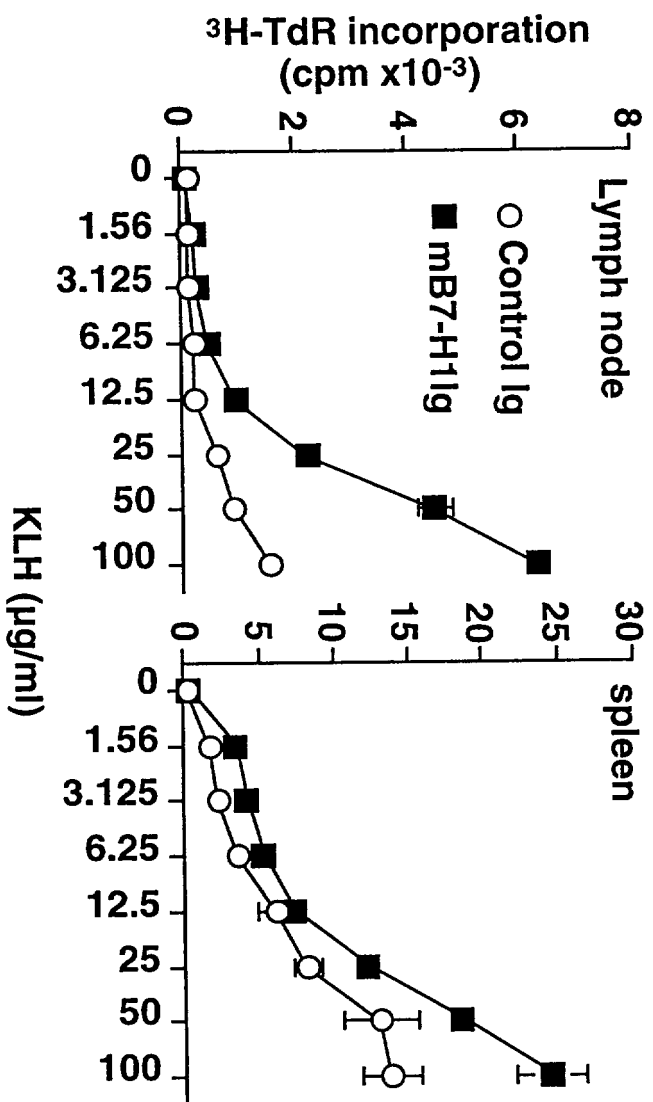


FIG. 21

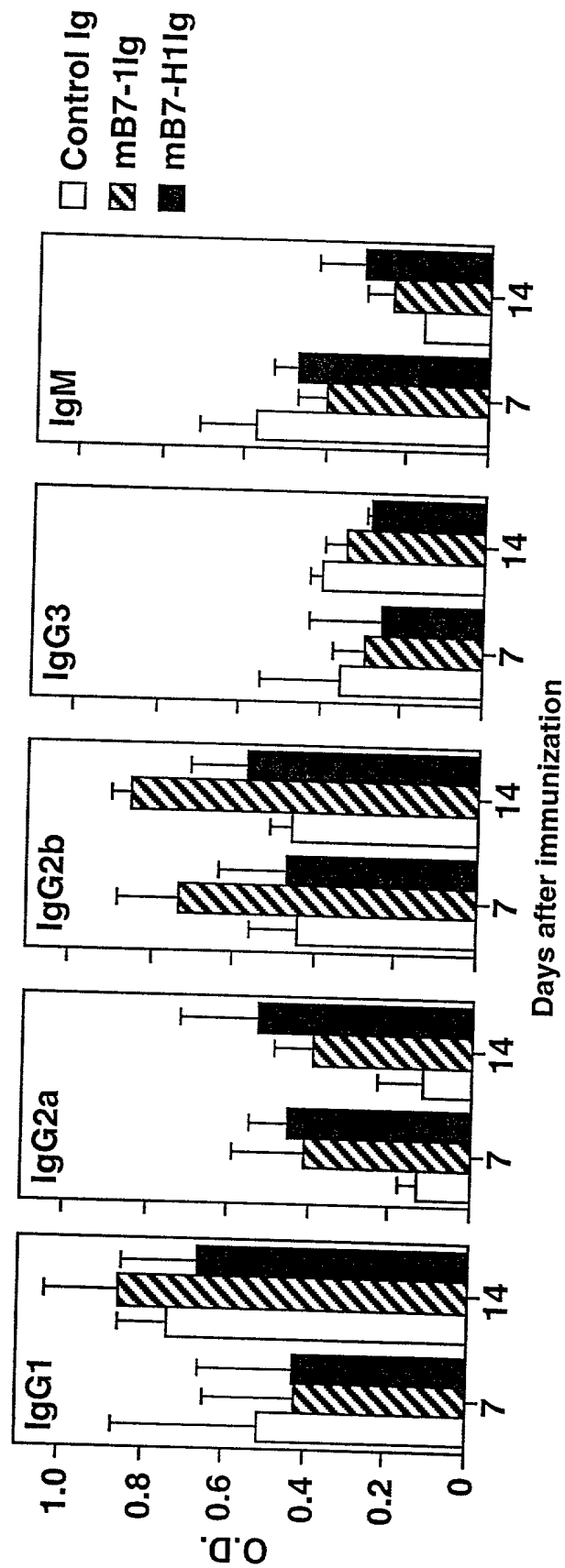


Fig. 22

FIG. 23a

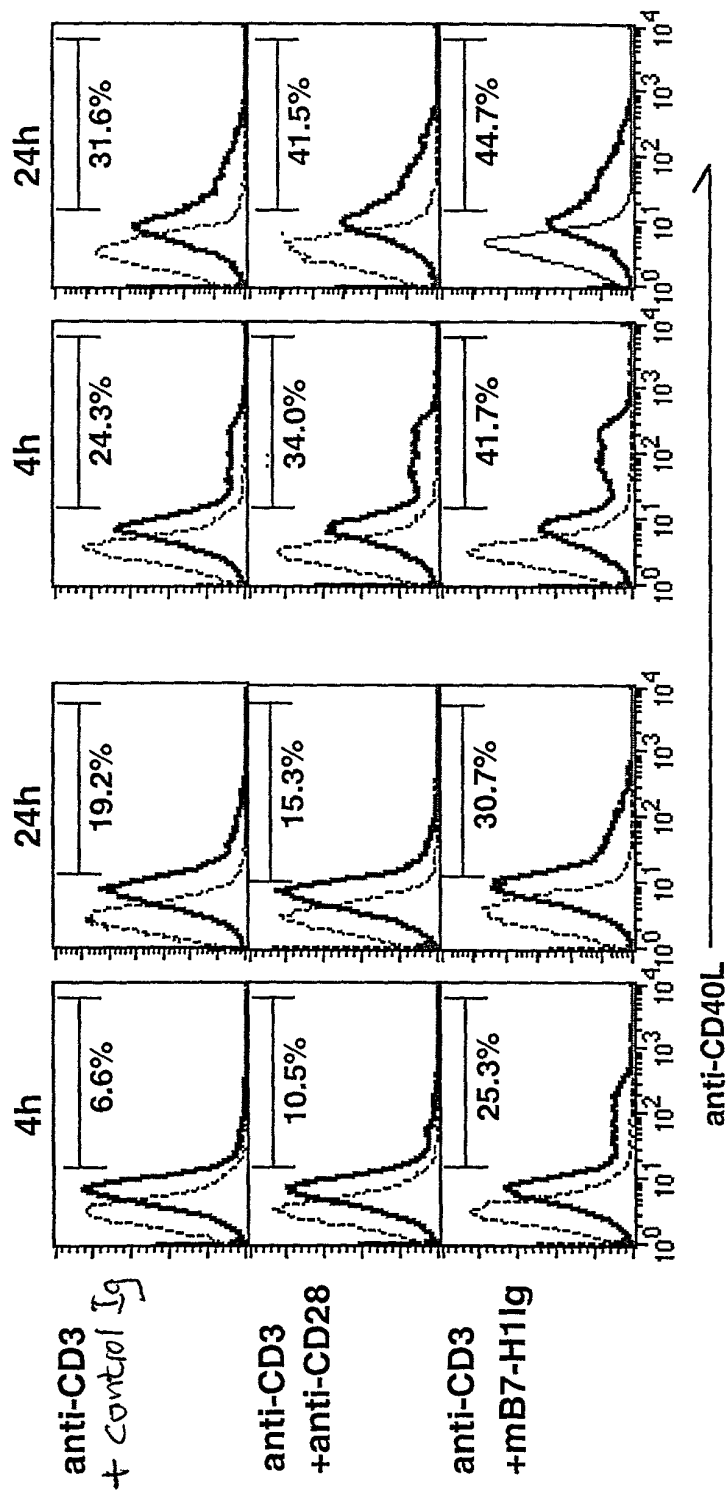


FIG. 23b

